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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EL 987 061 393 US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Alessandra	Bonci	Siena, Italy

Additional inventors are being named on the second *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Immunogenic Compositions for Chlamydia Trachomatis

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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OR

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Address					
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ENCLOSED APPLICATION PARTS *(check all that apply)*

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☐ Application Date Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☐ Applicant claims small entity status. See 37 CFR 1.27.

☑ A check or money order is enclosed to cover the filing fees.

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

SIGNATURE Rebecca M. Hale

Date June 1, 2004

REGISTRATION NO. 45,680 *(if appropriate)*

Docket Number: PP20662.004

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PROVISIONAL APPLICATION COVER SHEET
Additional Page

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Docket Number PP20662.004

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Number 2 of 2

PATENT
Atty. Dkt. No. PP20662.004

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

ALESSANDRA BONCI ET AL.

Serial No.:	Unassigned	Group Art Unit:	Unassigned
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FOR: IMMUNOGENIC COMPOSITIONS FOR CHLAMYDIA TRACHOMATIS

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I hereby certify that the attached Specification (_76_ pgs.), Provisional Application for Patent Cover Sheet (2 pgs.), Figures (17 sheets), Application Data Sheet (5 pgs), Check No. 8498 in the amount of $160.00 and Postal Receipt Card are being deposited with the United Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. §1.10 on the date indicated above is addressed to Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: 6/1/04

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IMMUNOGENIC COMPOSITIONS FOR *CHLAMYDIA TRACHOMATIS*

All documents cited herein are incorporated by reference in their entirety.

TECHNICAL FIELD

This invention is in the fields of immunology and vaccinology. In particular, it relates to antigens derived from *Chlamydia trachomatis* and their use in immunisation.

BACKGROUND ART

The *Chlamydiae* are obligate intracellular parasites of eukaryotic cells which are responsible for endemic sexually transmitted infections and various other disease syndromes. They occupy an exclusive eubacterial phylogenic branch, having no close relationship to any other known organisms.

Historically, the *Clamydiae* have been classified in their own order (*Chlamydiales*) made up of a single family (*Chlamydiaceae*) which in turn contains a single genus (*Chlamydia*, also referred to as *Chlamydophila*). More recently, this order has been divided into at least four families including *Chlamydiaceae*, *Parachlamydiaceae*, *Waddiaceae* and *Simkaniaceae*. In this more recent classification, the *Chlamydiaceae* family includes genuses of *Chlamydophila* and *Chlamydia*, *Chlamydia trachomatis* being a species within the *Chlamydia* genus. See Ref. i. A particular characteristic of the *Chlamydiae* is their unique life cycle, in which the bacterium alternates between two morphologically distinct forms: an extracellular infective form (elementary bodies, EB) and an intracellular non-infective form (reticulate bodies, RB). The life cycle is completed with the re-organization of RB into EB, which leave the disrupted host cell ready to infect further cells.

The genome sequences of at least five chlamydia or chlamydophila species are currently known – *C.trachomatis, C.pneumoniae, C.muridarum, C.pecorum* and *C.psittaci* (See Refs. ii, viii). The human serovariants ("serovars") of *C.trachomatis* are divided into two biovariants ("biovars"). Serovars A-K elicit epithelial infections primarily in the ocular tissue (A-C) or urogenital tract (D-K). Serovars L1, L2 and L3 are the agents of invasive lymphogranuloma venereum (LGV).

Although chlamydial infection itself causes disease, it is thought that the severity of symptoms in some patients is actually due to an aberrant host immune response. Failure to clear the infection results in persistent immune stimulation and, rather than helping the host, this results in chronic infection with severe consequences, including sterility and blindness. See, e.g., Ref. ix. In addition, the protection conferred by natural chlamydial infection is usually incomplete, transient, and strain-specific.

More than 4 million new cases of chlamydial sexually transmitted infections are diagnosed each year in the United States alone (8) and the cost of their treatment has been estimated in 4 billion dollars annually, with 80% attributed to infection and disease of women (9). Although chlamydial infections can be treated with several antibiotics, a majority of the female infections are asymptomatic, and antimicrobial therapy may be delayed or inadequate to prevent long term sequelae, expecially in countries with poor hygienic conditions. Multiple-antibiotic-resistant strains of Chlamydia have also been reported (Somani, et al., 2000). Furthermore it has been

suggested that antibiotic treatment could lead to the formation of aberrant forms of C. trachomatis that maybe reactivated later on (Hammerschlag M.R. 2002. The intracellular life of chlamydiae. Semin.Pediatr.Infect.Dis.13:239-248).

Unfortunately the major determinants of chlamydial pathogenesis are complicated and at present still unclear, mostly due to the intrinsic difficulty in working with this pathogen and the lack of adequate methods for its genetic manipulation. In particular very little is known about the antigenic composition of elementary body surface, that is an essential compartment in pathogen-host interactions, and likely to carry antigens able to elicit a protective immune response.

Due to the serious nature of the disease, there is a desire to provide suitable vaccines. These may be useful (a) for immunisation against chlamydial infection or against chlamydia-induced disease (prophylactic vaccination) or (b) for the eradication of an established chronic chlamydial infection (therapeutic vaccination). Being an intracellular parasite, however, the bacterium can generally evade antibody-mediated immune responses.

Various antigenic proteins have been described for *C.trachomatis*, and the cell surface in particular has been the target of detailed research. See, *e.g.*, Ref. x. These include, for instance, Pgp3 (Refs. xi, xii, and xiii), MOMP (Ref. xiv), Hsp60 (GroEL) (Ref. xv) and Hsp70 (DnaK-like) (Ref. xvi). Not all of these have proved to be effective vaccines, however, and further candidates have been identified. See Ref. xvii.

Vaccines against pathogens such as hepatitis B virus, diphtheria and tetanus typically contain a single protein antigen (*e.g.* the HBV surface antigen, or a tetanus toxoid). In contrast, acellular whooping cough vaccines typically have at least three *B.pertussis* proteins, and the Prevnar™ pneumococcal vaccine contains seven separate conjugated saccharide antigens. Other vaccines such as cellular pertussis vaccines, the measles vaccine, the inactivated polio vaccine (IPV) and meningococcal OMV vaccines are by their very nature complex mixtures of a large number of antigens. Whether protection can be elicited by a single antigen, a small number of defined antigens, or a complex mixture of undefined antigens, therefore depends on the pathogen in question.

It is an object of the invention to provide further and improved compositions for providing immunity against chlamydial disease and/or infection. The compositions are based on a combination of two or more (*e.g.* three or more) *C.trachomatis* antigens.

DISCLOSURE OF THE INVENTION

Within the ~900 proteins described for the *C.trachomatis* genome of reference v, Applicants have discovered a group of five *Chlamydia trachomatis* antigens that are particularly suitable for immunisation purposes, particularly when used in combinations. The invention therefore provides a composition comprising a combination of *Chlamydia trachomatis* antigens, said combination consisting of two, three, four or all five *Chlamydia trachomatis* antigens of a first antigen group, said first antigen group consisting of: (1) PepA (CT045); (2) LcrE (CT089); (3) ArtJ (CT381); (4) DnaK (CT396); and (5) CT398. These antigens are referred to herein as the 'first antigen group'.

Preferably, the composition of the invention comprises a combination of *Chlamydia trachomatis* antigens, said combination selected from the group consisting of: (1) PepA & LcrE; (2) PepA & ArtJ; (3) PepA & DnaK; (4) PepA & CT398; (5) LcrE & ArtJ; (6) LcrE & DnaK; (7) LcrE &

CT398; (8) ArtJ & DnaK; (9) ArtJ & CT398; (10) DnaK & CT398; (11) PepA, LcrE & ArtJ; (12) PepA, LcrE & DnaK; (13) PepA, LcrE & CT398; (14) PepA, ArtJ & DnaK; (15) PepA, ArtJ and CT398; (16) PepA, DnaK & CT398; (17) LcrE, ArtJ & DnaK; (18) LcrE, ArtJ & CT398; (19) LcrE, DnaK & CT398; (20) ArtJ, DnaK & CT398; (21) PepA, LcrE, ArtJ & DnaK; (22) PepA, LcrE, DnaK & CT398; (23) PepA, ArtJ, DnaK & CT398; (24) PepA, LcrE, ArtJ & CT398; (25) LcrE, ArtJ, DnaK & CT398; and (26) PepA, LcrE, ArtJ, DnaK & CT398. Preferably, the composition of *Chlamydia trachomatis* antigens consists of PepA, LcrE, ArtJ, DnaK & CT398.

The invention also provides for a slightly larger group of 13 *Chlamydia trachomatis* antigens that are particularly suitable for immunisation purposes, particularly when used in combinations. (This second antigen group includes the five *Chlamydia trachomatis* antigens of the first antigen group.) These 13 *Chlamydia trachomatis* antigens form a second antigen group of (1) PepA (CT045); (2) LcrE (CT089); (3) ArtJ (CT381); (4) DnaK (CT396); (5) CT398; (6) OmpH-like (CT242); (7) L7/L12 (CT316); (8) OmcA (CT444); (9) AtoS (CT467); (10) CT547; (11) Eno (CT587); (12) HtrA (CT823) and (13) MurG (CT761). These antigens are referred to herein as the 'second antigen group'.

The invention therefore provides a composition comprising a combination of *Chlamydia trachomatis* antigens, said combination selected from the group consisting of two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, or thirteen *Chlamydia trachomatis* antigens of the second antigen group. Preferably, the combination is selected from the group consisting of two, three, four or five *Chlamydia trachomatis* antigens of the second antigen group. Still more preferably, the combination consists of five *Chlamydia trachomatis* antigens of the second antigen group.

Each of the *Chlamydia trachomatis* antigens of the first and second antigen group are described in more detail below.

(1) PepA leucyl aminopeptidase A protein (CT045)

One example of a 'PepA' protein is disclosed as SEQ ID NO[s]: 71 & 72 in reference xvii {GenBank accession number: AAC67636, GI:3328437; 'CT045'; SEQ ID NO: 2 below}. It is believed to catalyse the removal of unsubstituted N-terminal amino acids from various polypeptides.

Preferred PepA proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 2; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 2, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These PepA proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 2. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 2. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 2. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain). The PepA protein may contain manganese ions.

SEQ ID NO: 2

MVLLYSQASWDKRSKADALVLPFWMKNSKAQEAAVVDEDYKLVYQNALSNFSGKKGETAFLFGNDHTKEQK
IVLLGLGKSEEVSGTTVLEAYAQATTVLRKAKCKTVNILLPTISQLRFSVEEFLTNLAAGVLSLNYNYPTYHKVD
TSLPFLEKVTVMGIVSKVGDKIFRKEESLFEGVYLTRDLVNTNADEVTPEKLAAVAKDLAGEFASLDVKILDRK
AILKEKMGLLAAVAKGAAVEPRFIVLDYQGKPKSKDRTVLIGKGVTFDSGGLDLKPGKAMITMKEDMAGAAT
VLGIFSALASLELPINVTGIIPATENAIGSAAYKMGDVYVGMTGLSVEIGSTDAEGRLILADAISYALKYCNPTRII
DFATLTGAMVVSLGESVAGFFANNDVLARDLAEASSETGEALWRMPLVEKYDQALHSDIADMKNIGSNRAGSI
TAALFLQRFLEDNPVAWAHLDIAGTAYHEKEELPYPKYATGFGVRCLIHYMEKFLSK

(2) LcrE low calcium response E protein (CT089)

One example of a 'LcrE' protein is disclosed as SEQ ID NO[s]: 61 & 62 in reference xvii {GenBank accession number: AAC67680, GI:3328485; 'CT089'; SEQ ID NO: 3 below}. Preferred LcrE proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 3; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 3, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These LcrE proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 3. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 3. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 3. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 3

MTASGGAGGLGSTQTVDVARAQAAAATQDAQEVIGSQEASEASMLKGCEDLINPAAATRIKKKGEKFESLEAR
RKPTADKAEKKSESTEEKGDTPLEDRFTEDLSEVSGEDFRGLKNSFDDDSSPDEILDALTSKFSDPTIKDLALDYL
IQTAPSDGKLKSTLIQAKHQLMSQNPQAIVGGRNVLLASETFASRANTSPSSLRSLYFQVTSSPSNCANLHQMLA
SYLPSEKTAVMEFLVNGMVADLKSEGPSIPPAKLQVYMTELSNLQALHSVNSFFDRNIGNLENSLKHEGHAPIPS
LTTGNLTKTFLQLVEDKFPSSSKAQKALNELVGPDTGPQTEVLNLFFRALNGCSPRIFSGAEKKQQLASVITNTL
DAINADNEDYPKPGDFPRSSFSSTPPHAPVPQSEIPTSPTSTQPPSP

(3) ArtJ arginine-binding protein (CT381)

One example of 'ArtJ' protein is disclosed as SEQ ID NO[s]: 105 & 106 in reference xvii {GenBank accession number: AAC67977, GI:3328806; 'CT381'; SEQ ID NO: 6 below}. Preferred ArtJ proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 6; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 6, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These ArtJ proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 6. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 6. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 6. Other fragments omit one or more domains of the

protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).
The ArtJ protein may be bound to a small molecule like arginine or another amino acid.

SEQ ID NO: 6

MCIKRKKTWIAFLAVVCSFCLTGCLKEGGDSNSEKFIVGTNATYPPFEFVDKRGEVVGFDIDLAREISNKLGKTL
DVREFSFDALILNLKQHRIDAVITGMSITPSRLKEILMIPYYGEEIKHLVLVFKGENKHPLPLTQYRSVAVQTGTY
QEAYLQSLSEVHIRSFDSTLEVLMEVMHGKSPVAVLEPSIAQVVLKDFPALSTATIDLPEDQWVLGYGIGVASDR
PALALKIEAAVQEIRKEGVLAELEQKWGLNN

(4) DnaK heat-shock protein 70 (chaperone)(CT396)

One example of 'DnaK' protein is disclosed as SEQ ID NO[s]: 107 & 108 in reference xvii {GenBank accession number: AAC67993, GI:3328822; 'CT396'; SEQ ID NO: 7 below}. Other sequences are disclosed in references xviii, xix and xx.
Preferred DnaK proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 7; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 7, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These DnaK proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 7. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 7. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 7. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain). The DnaK may be phosphorylated *e.g.* at a threonine or a tyrosine.

SEQ ID NO: 7

MSEKRKSNKIIGIDLGTTNSCVSVMEGGQPKVIASSEGTRTTPSIVAFKGGETLVGIPAKRQAVTNPEKTLASTKR
FIGRKFSEVESEIKTVPYKVAPNSKGDAVFDVEQKLYTPEEIGAQILMKMKETAEAYLGETVTEAVITVPAYFND
SQRASTKDAGRIAGLDVKRIIPEPTAAALAYGIDKEGDKKIAVFDLGGGTFDISILEIGDGVFEVLSTNGDTHLGG
DDFDGVIINWMLDEFKKQEGIDLSKDNMALQRLKDAAEKAKIELSGVSSTEINQPFITIDANGPKHLALTLTRAQ
FEHLASSLIERTKQPCAQALKDAKLSASDIDDVLLVGGMSRMPAVQAVVKEIFGKEPNKGVNPDEVVAIGAAIQ
GGVLGGEVKDVLLLDVIPLSLGIETLGGVMTPLVERNTTIPTQKKQIFSTAADNQPAVTIVVLQGERPMAKDNK
EIGRFDLTDIPPAPRGHPQIEVTFDIDANGILHVSAKDAASGREQKIRIEASSGLKEDEIQQMIRDAELHKEEDKQR
KEASDVKNEADGMIFRAEKAVKDYHDKIPAELVKEIEEHIEKVRQAIKEDASTTAIKAASDELSTHMQKIGEAM
QAQSASAAASSAANAQGGPNINSEDLKKHSFSTRPPAGGSASSTDNIEDADVEIVDKPE

(5) CT398 protein(Hypothetical Protein)

One example of 'CT398' protein is disclosed as SEQ ID NO[s]: 111 & 112 in reference xvii {GenBank accession number: AAC67995, GI:3328825; SEQ ID NO: 8 below}.
Preferred CT398 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 8; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 8, wherein *n* is 7 or more (*e.g.* 8,

10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These CT398 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 8. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 8. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 8. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 8

MHDALQSILAIQELDIKMIRLMRVKKEHQNELAKIQALKTDIRRKVEEKEQEMEKLKDQIKGGEKRIQEISDQIN
KLENQQAAVKKMDEFNALTQEMTAANKERRTLEHQLSDLMDKQAGSEDLLISLKESLSSTENSSSAIEEEIRENI
RKINEEGRSLLSQRTQLKETTDPELFSIYERLLNNKKDRVVVPIENRVCSGCHIALTPQHENLVRKQDHLVFCEH
CSRILYWQELQSPSAEGATTKRRRRRTAV

(6) OmpH-like outer membrane protein(CT242)

One example of 'OmpH-like' protein is disclosed as SEQ ID NO[s]: 57 & 58 in reference xvii {GenBank accession number: AAC67835, GI:3328652; 'CT242'; SEQ ID NO: 4 below}. A variant sequence is disclosed in reference xxi. Preferred OmpH-like proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 4; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 4, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These OmpH-like proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 4. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 4. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more; preferably 19 or more, to remove the signal peptide) from the N-terminus of SEQ ID NO: 4. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide as described above, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 4

MKKFLLLSLMSLSSLPTFAANSTGTIGIVNLRRCLEESALGKKESAEFEKMKNQFSNSMGKMEEELSSIYSKLQD
DDYMEGLSETAAAELRKKFEDLSAEYNTAQGQYYQILNQSNLKRMQKIMEEVKKASETVRIQEGLSVLLNEDI
VLSIDSSADKTDAVIKVLDDSFQNN

(7) L7/L12 ribosomal protein (CT316)

One example of 'L7/L12' protein is deposited in GenBank under accession number AAC67909 (GI:3328733; 'CT316'; SEQ ID NO: 5 below}. Preferred L7/L12 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 5; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 5, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These L7/L12 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 5. Preferred fragments of (b)

comprise an epitope from SEQ ID NO: 5. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 5. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain). The L7/L12 protein may be N-terminally modified.

SEQ ID NO: 5

MTTESLETLVEQLSGLTVLELSQLKKLLEEKWDVTAAAPVVAVAGAAAAGDAPASAEPTEFAVILEDVPSDKKI
GVLKVVREVTGLALKEAKEMTEGLPKTVKEKTSKSDAEDTVKKLQEAGAKAVAKGL

(8) OmcA cysteine-rich lipoprotein(CT444)

One example of 'OmcA' protein is disclosed as SEQ ID NO[s]: 127 & 128 in reference xvii {GenBank accession number: AAC68043, GI:3328876; 'CT444', 'Omp2A', 'Omp3'; SEQ ID NO: 9 below}. A variant sequence is disclosed in reference xxii. Preferred OmcA proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 9; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 9, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These OmcA proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 9. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 9. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more; preferably 18 or more to remove the signal peptide) from the N-terminus of SEQ ID NO: 9. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide as described above, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain). The protein may be lipidated (*e.g.* by a *N*-acyl diglyceride), and may thus have a N-terminal cysteine.

SEQ ID NO: 9

MKKTALLAALCSVVSLSSCCRIVDCCFEDPCAPIQCSPCESKKKDVDGGCNSCNGYVPACKPCGGDTHQDAKH
GPQARGIPVDGKCRQ

(9) AtoS two-component regulatory system sensor histidine kinase protein (CT467)

One example of 'AtoS' protein is disclosed as SEQ ID NO[s]: 129 & 130 in reference xvii {GenBank accession number: AAC68067, GI:3328901; 'CT467'; SEQ ID NO: 10 below}. Preferred AtoS proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 10; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 10, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These AtoS proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 10. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 10. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 10. Other fragments omit one or more domains of

the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 10

MPKIDTCDSCVSNTELLAIRTRVTQSYNEAQTILSSIPDGIFLLSESGEILICNPQARAILGIPEDIQLVTRMFHDFFP
DTFFGFSVQEALEKEVPPKTIRLTLSQELSQKEVEVFVRKNISHDFLFLLIRDRSDYRQLEQAIEKYRSISELGKIA
ATLAHEIRNPLTSISGFATLLKEELSSERHQRMLNVIIEGTRSLNSLVSSMLEYTKIQPLNLRSIDLQDFFSSLIPELS
LTFPSCTFRRTILSPIQRSIDPDRLRCVIWNLVKNAVEASDEEIFLELHEKGFSVINTGTLPPNIQEKLFIPFFTTKPQ
GNGLGLAEAHKIMRLHGGDLVVSTQDNRTTFTILWTPA

(10) CT547 protein(Hypothetical Protein)

One example of 'CT547' protein is disclosed as SEQ ID NO[s]: 151 & 152 in reference xvii {GenBank accession number: AAC67995, GI:3328825; SEQ ID NO: 11 below}.
Preferred CT547 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 11; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 11, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These CT547 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 11. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 11. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 11. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 11

MKVILRALCLFLVLPCGCYARVPSFEPFRGAIAPNRYTPKHSPELYFEMGDKYFQAKKFKQALLCFGMITHHFPE
HALHPKAQFLVGLCYLEMGHPDLADKALTQYQELADTEYSEQLFAIKYSIAQSFANGKRKNIVPLEGFPKLLKA
DTDALRIFEEIVTASSDADLKASALYAKGALLFDRKEYSEAIKTLKKVSLQFPSHSLSPESFTLIAKIHCLQALQEP
YNEQYLQDARMNAAALRKQHPNHPSNTEVENYIHHMCEAYASCLYSTGRFYEKKRKASSAKIYYSIALENFPD
TSYVAKCNKRLERLSKQMS

(11) Enolase (2-phosphoglycerate dehydratase) protein(CT587)

One example of an 'Eno' protein is disclosed as SEQ ID NO[s]: 189 & 190 in reference xvii {GenBank accession number: AAC68189, GI:3329030; 'CT587'; SEQ ID NO: 12 below}.
Preferred Eno proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 12; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 12, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Eno proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 12. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 12. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 12. Other fragments omit one or more domains of

the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain). The Eno protein may contain magnesium ions, and may be in the form of a homodimer.

SEQ ID NO: 12

MFDVVISDIEAREILDSRGYPTLCVKVITNTGTFGEACVPSGASTGIKEALELRDKDPKRYQGKGVLQAISNVEK
VLMPALQGFSVFDQITADAIMIDADGTPNKEKLGANAILGVSLALAKAAANTLQRPLYRYLGGSFSHVLPCPM
MNLINGGMHATNGLQFQEFMIRPISAPSLTEAVRMGAEVFNALKKILQNRQLATGVGDEGGFAPNLASNAEAL
DLLLTAIETAGFTPREDISLALDCAASSFYNTQDKTYDGKSYADQVGILAELCEHYPIDSIEDGLAEEDFEGWKL
LSETLGDRVQLVGDDLFVTNSALIAEGIAQGLANAVLIKPNQIGTLTETAEAIRLATIQGYATILSHRSGETEDTTI
ADLAVAFNTGQIKTGSLSRSERIAKYNRLMAIEEEMGPEALFQDSNPFSKA

(12) HrtA DO protease protein(CT823)

One example of an 'HrtA' protein is disclosed as SEQ ID NO[s]: 229 & 230 in reference xvii {GenBank accession number: AAC68420, GI:3329293; 'CT823'; SEQ ID NO: 13 below}. Preferred HrtA proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 13; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 13, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These HrtA proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 13. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 13. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more; preferably at least 16 to remove the signal peptide) from the N-terminus of SEQ ID NO: 13. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide as described above, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain). In relation to SEQ ID NO: 13, distinct domains are residues: 1-16; 17-497; 128-289; 290-381; 394-485; and 394-497.

SEQ ID NO: 13

MMKRLLCVLLSTSVFSSPMLGYSASKKDSKADICLAVSSGDQEVSQEDLLKEVSRGFSRVAAKATPGVVYIENF
PKTGNQAIASPGNKRGFQENPFDYFNDEFFNRFFGLPSHREQQRPQQRDAVRGTGFIVSEDGYVVTNHHVVEDA
GKIHVTLHDGQKYTAKIVGLDPKTDLAVIKIQAEKLPFLTFGNSDQLQIGDWAIAIGNPFGLQATVTVGVISAKG
RNQLHIVDFEDFIQTDAAINPGNSGGPLLNINGQVIGVNTAIVSGSGGYIGIGFAIPSLMAKRVIDQLISDGQVTRG
FLGVTLQPIDSELATCYKLEKVYGALVTDVVKGSPAEKAGLRQEDVIVAYNGKEVESLSALRNAISLMMPGTR
VVLKIVREGKTIEIPVTVTQIPTEDGVSALQKMGVRVQNITPEICKKLGLAADTRGILVVAVEAGSPAASAGVAP
GQLILAVNRQRVASVEELNQVLKNSKGENVLLMVSQGDVVRFIVLKSDE

(13) MurG peptidoglycan transferase protein(CT761)

One example of a 'MurG' protein is disclosed as SEQ ID NO[s]: 217 & 218 in reference xvii {GenBank accession number: AAC68356, GI:3329223; 'CT761'; SEQ ID NO: 14 below} It is a UDP-N-acetylglucosamine-N-acetylmuramyl-(pentapeptide)pyrophosphoryl-undecaprenol N-acetylglucosamine transferase. Preferred MurG proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 14;

and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 14, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These MurG proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 14. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 14. Other preferred fragments lack one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (*e.g.* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 14. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide as described above, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain). The MurG may be lipidated *e.g.* with undecaprenyl.

SEQ ID NO: 14

MKKINKIVLAVGGTGGHIIPALAARETFIHEDIEVLLLGKGLAHFLGDDSEVAYCDIPSGSPFSLRVNRMFSGAKQ
LYKGYVAALQKIRDFTPDLAIGFGSYHSLPAMLASIRSRIPLFLHEQNIVPGKVNKLFSRFAKGVGMSFAAAGEH
FHCRAEEVFLPIRKLSEQIVFPGASPVICVVGGSQGAKILNDVVPKALARIRESYSNLYVHHIVGPKGDLQAVSQ
VYQDAGINHTVTAFDHNMLGVLQASDLVISRSGATMLNELLWVQVPAILIPYPGAYGHQEVNAKFFTHTVGGG
TMILQKYLTEESLSKQVLLALDPATSENRRKAMLSAQQKKSFKSLYQFICESL

The immunogenicity of other known *Chlamydia trachomatis* antigens may be improved by combination with two or more *Chlamydia trachomatis* antigens from either the first antigen group or the second antigen group. Such other known *Chlamydia trachomatis* antigens include a third antigen group consisting of (1) PGP3, (2) one or more PMP, (3) MOMP (CT681), (4) Cap1 (CT529); (5) GroEL-like hsp60 protein (Omp2); and (6) 60 kDa Cysteine rich protein (omcB). These antigens are referred to herein as the "third antigen group".

The invention thus includes a composition comprising a combination of *Chlamydia trachomatis* antigens, said combination selected from the group consisting of two, three, four, or five *Chlamydia trachomatis* antigens of the first antigen group and one, two, three, four, five or six *Chlamydia trachomatis* antigens of the third antigen group. Preferably, the combination is selected from the group consisting of three, four, or five *Chlamydia trachomatis* antigens from the first antigen group and three, four, or five *Chlamydia trachomatis* antigens from the third antigen group. Still more preferably, the combination consists of five *Chlamydia trachomatis* antigens from the first antigen group and three, four or five *Chlamydia trachomatis* antigens from the third antigen group.

The invention further includes a composition comprising a combination of *Chlamydia trachomatis* antigens, said combination selected from the group consisting of two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or thirteen *Chlamydia trachomatis* antigens of the second antigen group and one, two, three, four, five or six *Chlamydia trachomatis* antigens of the third antigen group. Preferably, the combination is selected from the group consisting of three, four, or five *Chlamydia trachomatis* antigens from the second antigen group and three, four or five *Chlamydia trachomatis* from the third antigen group. Still more preferably, the combination consists of five *Chlamydia trachomatis* antigens from the second antigen group and three, four or five *Chlamydia trachomatis* antigens of the third antigen group.

In either of the above combinations, preferably the *Chlamydia trachomatis* antigens from the third antigen group include Cap 1. Or, alternatively, in either of the above combinations, preferably the *Chlamydia trachomatis* antigens from the third antigen group include MOMP.

Each of the *Chlamydia trachomatis* antigens of the third antigen group are described in more detail below.

(1) Plasmid Encoded Protein (PGP3)

One example of PGP3 sequence is disclosed in, for example, at Genbank entry GI 121541. Immunization with pgp3 is discussed in Ref. xxiii and xxiv. One example of a PGP3 protein is set forth below as SEQ ID NO: 15. Preferred PGP3 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 15; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 15, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These PGP3 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 15. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 15. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 15. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 15

MGNSGFYLYNTQNCVFADNIKVGQMTEPLKDQQIILGTTSTPVAAKMTASDGISLTVSN
NPSTNASITIGLDAEKAYQLILEKLGDQILGGIADTIVDSTVQDILDKITTDPSLGLLKAFN
NFPITNKIQCNGLFTPRNIETLLGGTEIGKFTVTPKSSGSMFLVSADIIASRMEGGVVLALV
REGDSKPYAISYGYSSGVPNLCSLRTRIINTGLTPTTYSLRVGGLESGVVWVNALSNGND
ILGITNTSNVSFLEVIPQTNA

(2) Polymorphic Membrane Proteins (PMP)

A family of nine *Chlamydia trachomatis* genes encoding predicted polymorphic membrane proteins (PMP) have been identified (*pmpA* to *pmpI*). See Ref. xxv, specifically Figure 1. Examples of Amino acid sequences of the PMP genes are set forth as SEQ ID NOS: 16 – 24. (These sequences can also be found at Genbank Ref. Nos. GI 15605137 (*pmpA*), 15605138 (*pmpB*), 15605139 (*pmpC*), 15605546 (*pmpD*), 15605605 (*pmpE*), 15605606 (*pmpF*), 15605607 (*pmpG*), 15605608 (*pmpH*), and 15605610 (*pmpH*)). These PMP genes encode relatively large proteins (90 to 187 kDa in mass). The majority of these PMP proteins are predicted to be outer membrane proteins, and are thus also referred to as Predicted Outer Membrane Proteins. As used herein, PMP refers to one or more of the *Chlamydia trachomatis* pmp proteins (*pmpA* to *pmpI*) or an immunogenic fragment thereof. Preferably, the PMP protein used in the invention is *pmpE* or *pmpI*. Preferably, the PMP protein used in the invention comprises one or more of the fragments of *pmpE* or *pmpI* identified in International Patent Application PCT/US01/30345 (WO 02/28998) in Table 1 on page 20 (preferred fragments of *pmpE*) or Table 2 on page 21 (preferred fragments of *pmpI*).

Preferred PMP proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to one of the polypeptide sequences set forth as SEQ ID NOS: 16 - 24; and/or (b) which is a fragment of at least *n* consecutive amino acids of one of the polypeptide sequences set forth as SEQ ID NOS: 16 - 24, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These PMP

proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of the polypeptide sequences set forth as SEQ ID NOS: 16 - 24. Preferred fragments of (b) comprise an epitope from one of the polypeptide sequences set forth as SEQ ID NOS: 16 - 24. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of one of the polypeptide sequences set forth as SEQ ID NOS: 16 - 24. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 16 (pmpA)

MNRVIEIHAHYDQRQLSQSPNTNFLVHHPYLTLIPKFLLGALIVYAPYSFAEMELAISGHKQGKDRDTFT
MISSCPEGTNYIINRKLILSDFSLLNKVSSGGAFRNLAGKISFLGKNSSASIHFKHININGFGAGVFSES
SIEFTDLRKLVAFGSESTGGIFTAKEDISFKNNHHIAFRNNITKGNGGVIQLQGDMKGSVSFVDQRGAII
FTNNQAVTSSSMKHSGRGGAISGDFAGSRILFLNNQQITFEGNSAVHGGAIYNKNGLVEFLGNAGPLAFKE
NTTIANGGAIYTSNFKANQQTSPILFSQNHANKKGGAIYAQYVNLEQNQDTIRFEKNTAKEGGGAITSSQCS
ITAHNTIIFSDNAAGDLGGGAILLEGKKPSLTLIAHSGNIAFSGNTMLHITKKASLDRHNSILIKEA
PYKIQLAANKNHSIHFFDPVMALSASSSPIQINAPEYETPFFSPKGMIVFSGANLLDDAREDVANRTSIF
NQPVHLYNGTLSIENGAHLIVQSFKQTGGRISLSPGSSLALYTMNSFFHGNISSKEPLEINGLSFGVDIS
PSNLQAEIRAGNAPLRLSGSPSIHDPEGLFYENRDTAASPYQMEILLTSDKIVDISKFTTDSLVTNKQSG
FQGAWHFSWQPNTINNTKQKILRASWLPTGEYVLESNRVGRAVPNSLWSTFLLLQTASHNLGDHLCNNRS
LIPTSYFGVLIGGTGAEMSTHSSEEESFISRLGATGTSIIRLTPSLTLSGGGSHMFGDSFVADLPEHITS
EGIVQNVGLTHVWGPLTVNSTLCAALDHNAMVRICSKKDHTYGKWDTFGMRGTLGASYTFLEYDQTMR
VFSFANIEATNILQRAFTETGYNPRSFSKTKLLNIAIPIGIGYEFCLGNSSFALLGKGSIGYSRDIKRENPS
TLAHLAMNDFAWTTNGCSVPTSAHTLANQLILRYKACSLYITAYTINREGKNLSNSLSCGGYVGF

SEQ ID NO: 17 (pmpB)

MKWLSATAVFAAVLPSVSGFCFPEPKELNFSRVGTSSSTTFTETVGEAGAEYIVSGNASFTKFTNIPTTD
TTTPTNSNSSSSNGETASVSEDSDSTTTTPDPKGGGAFYNAHSGVLSFMTRSGTEGSLTLSEIKITGEGG
AIFSQGELLFTDLTGLTIQNNLSQLSGGAIFGESTISLSGITKATFSSNSAEVPAPVKKPTEPKAQTASE
TSGSSSSSGNDSVSSPSSSRAEPAAANLQSHFICATATPAAQTDTETSTPSHKPGSGGAIYAKGDLTIAD
SQEVLFSINKATKDGGAIFAEKDVSFENITSLKVQTNGAEEKGGAIYAKGDLSIQSSKQSLFNSNYSKQG
GGALYVEGDINFQDLEEIRIKYNKAGTFETKKITLPKAQASAGNADAWASSSPQSGSGATTVSNSGDSSS
GSDSDTSETVPATAKGGGLYTDKNLSITNITGIIEIANNKATDVGGGAYVKGTLTCENSHRLQFLKNSSD
KQGGGIYGEDNITLSNLTGKTLFQENTAKEEGGGLFIKGTDKALTMTGLDSFCLINNTSEKHGGGAFVTK
EISQTYTSDVETIPGITPVHGETVITGNKSTGGNGGGVCTKRLALSNLQSISISGNSAAENGGGAHTCPD
SFPTADTAEQPAAASAATSTPESAPVVSTALSTPSSSTVSSLTLLAASSQASPATSNKETQDPNADTDLL
IDYVVDTTISKNTAKKGGGIYAKKAKMSRIDQLNISENSATEIGGGICCKESLELDALVSLSVTENLVGK
EGGGLHAKTVNISNLKSGFSFSNNKANSSSTGVATTASAPAAAAASLQAAAAAVPSSPATPTYSGVVGGA
IYGEKVTFSQCSGTCQFSGNQAIDNNPSQSSLNVQGGAIYAKTSLSIGSSDAGTSYIFSGNSVSTGKSQT
TGQIAGGAIYSPTVTLNCPATFSNNTASMATPKTSSEDGSSGNSIKDTIGGAIAGTAITLSGVSRFSGNT
ADLGAAIGTLANANTPSATSGSQNSITEKITLENGSFIFERNQANKRGAIYSPSVSIKGNNITFNQNTST
HDGSAIYFTKDATIESLGSVLFTGNNVTATQASSATSGQNTNTANYGAAIFGDPGTTQSSQTDAILTLLA
SSGNITFSNNSLQNNQGDTPASKFCSIAGYVKLSLQAAKGKTISFFDCVHTSTKKIGSTQNVYETLDINK
EENSNPYTGTIVFSSELHENKSYIPQNAILHNGTLVLKEKTELHVVSFEQKEGSKLIMKPGAVLSNQNIA
NGALVINGLTIDLSSMGTPQAGEIFSPPELRIVATTSSASGGSGVSSSIPTNPKRISAAAPSGSAATTPT
MSENKVFLTGDLTLIDPNGNFYQNPMLGSDLDVPLIKLPTNTSDVQVYDLTLSGDLFPQKGYMGTWTLDS
NPQTGKLQARWTFDTYRRWVYIPRDNHFYANSILGSQNSMIVVKQGLINNMLNNARFDDIAYNNFWVSG
VGTFLAQQGTPLSEEFSYYSRGTSVAIDAKPRQDFILGAAFSKMVGKTKAIKKMHNYFHKGSEYSYQASVY
GGKFLYFLLNKQHGWALPFLIQGVVSYGHIKHDTTTLYPSIHERNKGDWEDLGWLADLRISMDLKEPSKD
SSKRITVYGELEYSSIRQKQFTEIDYDPRHFDDCAYRNLSLPVGCAVEGAIMNCNILMYNKLALAYMPSI
YRNNPVCKYRVLSSNEAGQVICGVPTRTSARAEYSTQLYLGPFWTLYGNYTIDVGMYTLSQMTSCGARMI
F

SEQ ID NO: 18 (pmpC)

MKFMSATAVFAAALSSVTEASSIQDQIKNTDCNVSKLGYSTSQAFTDMMLADNTEYRAADSVSFYDFSTS
SRLPRKHLSSSSEASPTTEGVSSSSSGETDEKTEEELDNGGIIYAREKLTISESQDSLSNQSIELHDNSI
FFGEGEVIFDHRVALKNGGAIYGEKEVVFENIKSLLVEVNIAVEKGGSVYAKERVSLENVTEATFSSNGG
EQGGGGIYSEQDMLISDCNNVHFQGNAAGATAVKQCLDEEMIVLLAECVDSLSEDTLDSTPETEQTESNG
NQDGSSETEDTQVSESPESTPSPDDVLGKGGGIYTEKSLTITGITGTIDFVSNIATDSGAGVFTKENLSC
TNTNSLQFLKNSAGQHGGGAYVTQTMSVTNTTSESITTPPLIGEVIFSENTAKGHGGGICTNKLSLSNLK
TVTLTKNSAKESGGAIFTDLASIPITDTPESSTPSSSSPASTPEVVASAKINRFFASTAKPAAPSLTEAE
SDQTDQTETSDTNSDIDVSIENILNVAINQNTSAKKGGAIYGKKAKLSRINNLELSGNSSQDVGGGLCLT
ESVEFDAIGSLLSHYNSAAKEGGAIHSKTVTLSNLKSTFTFADNTVKAIVESTPEAPEEIPPVEGEESTA
TEDPNSNTEGSSANTNLEGSQGDTADTGTGDVNNESQDTSDTGNAESEEQLQDSTQSNEENTLPNSNIDQ
SNENTDESSDSHTEEITDESVSSSSESGSSTPQDGGAASSGAPSGDQSISANACLAKSYAASTDSSPVSN
SSGSEEPVTSSSDSDVTASSDNPDSSSSGDSAGDSEEPTEPEAGSTTETLTLIGGGAIYGETVKIENFSG
QGIFSGNKAIDNTTEGSSSKSDVLGGAVYAKTLFNLDSGSSRRTVTFSGNTVSSQSTTGQVAGGAIYSPT
VTIATPVVFSKNSATNNANNTTDTQRKDTFGGAIGATSAVSLSGGAHFLENVADLGSAIGLVPGTQNTET
VKLESGSYYFEKNKALKRATIYAPVVSIKAYTATFNQNRSLEEGSAIYFTKEASIESLGSVLFTGNLVTL
TLSTTTEGTPATTSGDVTKYGAAIFGQIASSNGSQTDNLPLKLIASGGNICFRNNEYRPTSSDTGTSTFC
SIAGDVKLTMQAAKGKTISFFDAIRTSTKKTGTQATAYDTLDINKSEDSETVNSAFTGTILFSSELHENK
SYIPQNVVLHSGSLVLKPNTELHVISFEQKEGSSLVMTPGSVLSNQTVADGALVINNMTIDLSSVEKNGI
AEGNIFTPPELRIIDTTTGGSGGTPSTDSESNQNSDDTEEQNNNDASNQGESANGSSSPAVAAAHTSRTR
NFAAAAATATPTTTPTATTTTSNQVILGGEIKLIDPNGTFFQNPALRSDQQISLLVLPTDSSKMQAQKIVL
TGDIAPQKGYTGTLTLDPDQLQNGTISVLWKFDSYRQWAYVPRDNHFYANSILGSQMLMVTVKQGLLND
KMNLARFEEVSYNNLWISGLGTMLSQVGTPTSEEFTYYSRGASVALDAKPAHDVIVGAAFSKMIGKTKSL
KRENNYTHKGSEYSYQASVYGGKPFHFVINKKTEKSLPLLLQGVISYGYIKHDTVTHYPTIRERNKGEWED
LGWLTALRVSSVLRTPAQGDTKRITVYGELEYSSIRQKQFTETEYDPRYFDNCTYRNLAIPMGLAFEGEL
SGNDILMYNRFSVAYMLSIYRNSPTCKYQVLSSGEGGEIICGVPTRNSARGEYSTQLYLGPLWTLYGSYT
IEADAHTLAHMMNCGARMTF

SEQ ID NO: 19 (pmpD)(CT812)

MSSEKDIKSTCSKFSLSVVAAILASVSGLASCVDLHAGGQSVNELVYVGPQAVLLLDQIRDLFVGSKDSQ
AEGQYRLIVGDPSSFQEKDADTLPGKVEQSTLFSVTNPVVFQGVDQQDQVSSQGLICSFTSSNLDSPRDG
ESFLGIAFVGDSSKAGITLTDVKASLSGAALYSTEDLIFEKIKGGLEFASCSSLEQGGACAAQSILIHDC
QGLQVKHCTTAVNAEGSSANDHLGFGGGAFFVTGSLSGEKSLYMPAGDMVVANCDGAISFEGNSANFAN
GGAIAASGKVLFVANDKKTSFIENRALSGGAIAASSDIAFQNCAELVFKGNCAIGTEDKGSLGGGAISSLG
TVLLQGNHGITCDKNESASQGGAIFGKNCQISDNEGPVVFRDSTACLGGGAIAAQEIVSIQNNQAGISFE
GGKASFGGGIACGSFSSAGGASVLGTIDISKNLGAISFSRTLCTTSDLGQMEYQGGGALFGENISLSENA
GVLTFKDNIVKTFASNGKILGGGAILATGKVEITNNSEGISFTGNARAPQALPTQEEFPLFSKKEGRPLS
SGYSGGGAILGREVAILHNAAVVFEQNRLQCSEEEATLLGCCGGGAVHGMDSTSIVGNSSVRFGNNYAMG
QGVSGGALLSKTVQLAGNGSVDFSRNIASLGGGALQASEGNCELVDNGYVLFRDNRGRVYGGAISCLRGD
VVISGNKGRVEFKDNIATRLYVEETVEKVEEVEPAPEQKDNNELSFLGRAEQSFITAANQALFASEDGDL
SPESSISSEELAKRRECAGGAIFAKRVRIVDNQEAVVFSNNFSDIYGGAIFTGSLREEDKLDGQIPEVLI
SGNAGDVVFSGNSSKRDEHLPHTGGGAICTQNLTISQNTGNVLFYNNVACSGGAVRIEDHGNVLLEAFGG
DIVFKGNSSFRAQGSDAIYFAGKESHITALNATEGHAIVFHDALVFENLEERKSAEVLLINSRENPGYTG
SIRFLEAESKVPQCIHVQQGSLELLNGATLCSYGFKQDAGAKLVLAAGAKLKILDSGTPVQQGHAISKPE
AEIESSSEPEGAHSLWIAKNAQTTVPMVDIHTISVDLASFSSSQQEGTVEAPQVIVPGGSYVRSGELNLE
LVNTTGTGYENHALLKNEAKVPLMSFVASGDEASAEISNLSVSDLQIHVVTPEIEEDTYGHMGDWSEAKI
QDGTLVISWNPTGYRLDPQKAGALVFNALWEEGAVLSALKNARFAHNLTAQRMEFDYSTNVWGFAFGGF
RTLSAENLVAIDGYKGAYGGASAGVDIQLMEDFVLGVSGAAFLGKMDSQKFDAEVSRKGVVGSVYTGFL
AGSWFFKGQYSLGETQNDMKTRYGVLGESSASWTSRGVLADALVEYRSLVGPVRPTFYALHFNPYVEVS
YASMKFPGFTEQGREARSFEDASLTNITIPLGMKFELAFIKGQFSEVNSLGISYAWEAYRKVEGGAVQLLEA
GFDWEGAPMDLPRQELRVALENNTEWSSYFSTVLGLTAFCGGFTSTDSKLGYEANTGLRLIF

SEQ ID NO: 20 (pmpE)

MKKAFFFFLIGNSLSGLAREVPSRIFLMPNSVPDPTKESLSNKISLTGDTHNLTNCYLDNLRYILAILQK
TPNEGAAVTITDYLSFFDTQKEGIYFAKNLTPESGGAIGYASPNSPTVEIRDTIGPVIFENNTCCRLFTW
RNPYAADKIREGGAIHAQNLYINHNHDVVGFMKNFSYVQGGAISTANTFVVSENQSCFLFMDNICIQTNT

AGKGGAIYAGTSNSFESNNCDLFFINNACCAGGAIFSPICSLTGNRGNIVFYNNRCFKNVETASSEASDG
GAIKVTTRLDVTGNRGRIFFSDNITKNYGGAIYAPVVTLVDNGPTYFINNIANNKGGAIYIDGTSNSKIS
ADRHAIIFNENIVTNVTNANGTSTSANPPRRNAITVASSSGEILLGAGSSQNLIFYDPIEVSNAGVSVSF
NKEADQTGSVVFSGATVNSADFHQRNLQTKTPAPLTLSNGFLCIEDHAQLTVNRFTQTGGVVSLGNGAVL
SCYKNGTGDSASNASITLKHIGLNLSSILKSGAEIPLLWVEPTNNSNNYTADTAATFSLSDVKLSLIDDY
GNSPYESTDLTHALSSQPMLSISEASDNQLQSENIDFSGLNVPHYGWQGLWTWGWAKTQDPEPASSATIT
DPQKANRFHRTLLLTWLPAGYVPSPKHRSPLIANTLWGNMLLATESLKNSAELTPSGHPFWGITGGGLGM
MVYQDPRENHPGFHMRSSGYSAGMIAGQTHTFSLKFSQTYTKLNERYAKNNVSSKNYSCQGEMLFSLQE
GFLLTKLVGLYSYGDHNCHHFYTQGENLTSQGTFRSQTMGGAVFFDLPMKPFGSTHILTAPFLGALGIYSS
LSHFTEVGAYPRSFSTKTPLINVLVPIGVKGSFMNATHRPQAWTVELAYQPVLYRQEPGIAAQLLASKGI
WFGSGSPSSRHAMSYKISQQTQPLSWLTLHFQYHGFYSSSTFCNYLNGEIALRF

SEQ ID NO: 21 (pmpF)

MIKRTSLSFACLSFFYLSTISILQANETDTLQFRRFTFSDREIQFVLDPASLITAQNIVLSNLQSNGTGA
CTISGNTQTQIFSNSVNTTADSGGAFDMVTTSFTASDNANLLFCNNYCTHNKGGGAIRSGGPIRFLNNQD
VLFYNNISAGAKYVGTGDHNEKNRGGALYATTITLTGNRTLAFINNMSGDCGGAISADTQISITDTVKGI
LFENNHTLNHIPYTQAENMARGGAICSRRDLCSISNNSGPIVFNYNQGGKGGAISATRCVIDNNKERIIF
SNNSSLGWSQSSSASNGGAIQTTQGFTLRNNKGSIYFDSNTATHAGGAINCGYIDIRDNGPVYFLNNSAA
WGAAFNLSKPRSATNYIHTGTGDIVFNNNVVFTLDGNLLGKRKLFHINNNEITPYTLSLGAKKDTRIYFY
DLFQWERVKENTSNNPPSPTSRNTITVNPETEFSGAVVFSYNQMSSDIRTLMGKEHNYIKEAPTTLKFGT
LAIEDDAELEIFNIPFTQNPTSLLALGSGATLTVGKHGKLNITNLGVILPIILKEGKSPPCIRVNPQDMT
QNTGTGQTPSSTSSISTPMIIFNGRLSIVDENYESVYDSMDLSRGKAEQLILSIETTNDGQLDSNWQSSL
NTSLLSPPHYGYQGLWTPNWITTTYTITLNNNSSAPTSATSIAEQKKTSETFTPSNTTTASIPNIKASAG
SGSGSASNSGEVTITKHTLVVNWAPVGYIVDPIRRGDLIANSLVHSGRNMTMGLRSLLPDNSWFALQGAA
TTLFTKQQKRLSYHGYSSASKGYTVSSQASGAHGHKFLLSFSQSSDKMKEKETNNRLSSRYYLSALCFEH
PMFDRIALIGAAACNYGTHNMRSFYGTKKSSKGKFHSTTLGASLRCELRDSMPLRSIMLTPFAQALFSRT
EPASIRESGDLARLFTLEQAHTAVVSPIGIKGAYSSDTWPTLSWEMELAYQPTLYWKRPLLNTLLIQNNG
SWVTTNTPLAKHSFYGRGSHSLKFSHLKLFANYQAEVATSTVSHYINAGGALVF

SEQ ID NO: 22 (pmpG)

MQTSFHKFFLSMILAYSCCSLSGGGYAAEIMIPQGIYDGETLTVSFPYTVIGDPSGTTVFSAGELTLKNL
DNSIAALPLSCFGNLLGSFTVLGRGHSLTFENIRTSTNGAALSDSANSGLFTIEGFKELSFSNCNSLLAV
LPAATTNNGSQTPTTTSTPSNGTIYSKTDLLLLNNEKFSFYSNLVSGDGGAIDAKSLTVQGISKLCVFQE
NTAQADGGACQVVTSFSAMANEAPIAFIANVAGVRGGGIAAVQDGQQGVSSSTSTEDPVVSFSRNTAVEF
DGNVARVGGGIYSYGNVAFLNNGKTLFLNNVASPVYIAAEQPTNGQASNTSDNYGDGGAIFCKNGAQAA
GSNNSGSVSFDGEGVVFFSSNVAAGKGGAIYAKKLSVANCGPVQFLGNIANDGGAIYLGESGELSLSADYG
DIIFDGNLKRTAKENAADVNGVTVSSQAISMGSGGKITTLRAKAGHQILFNDPIEMANGNNQPAQSSEPL
KINDGEGYTGDIVFANGNSTLYQNVTIEQGRIVLREKAKLSVNSLSQTGGSLYMEAGSTLDFVTPQPPQQ
PPAANQLITLSNLHLSLSSLLANNAVTNPPTNPPAQDSHPAIIGSTTAGSVTISGPIFFEDLDDTAYDRY
DWLGSNQKIDVLKLQLGTQPSANAPSDLTLGNEMPKYGYQGSWKLAWDPNTANNGPYTLKATWTKTGY
NPGPERVASLVPNSLWGSILDIRSAHSAIQASVDGRSYCRGLWVSGVSNFFYHDRDALGQGYRYISGGYSL
GANSYFGSSMFGLAFTEVFGRSKDYVVCRSNHHACIGSVYLSTKQALCGSYLFGDAFIRASYGFGNQHMK
TSYTFAEESDVRWDNNCLVGEIGVGLPIVITPSKLYLNELRPFVQAEFSYADHESFTEEGDQARAFRSGHL
MNLSVPVGVKFDRCSSTHPNKYSFMGAYICDAYRTISGTQTTLLSHQETWTTDAFHLARHGVIVRGSMYA
SLTSNIEVYGHGRYEYRDTSRGYGLSAGSKVRF

SEQ ID NO: 23 (pmpH)

MPFSLRSTSFCFLACLCSYSYGFASSPQVLTPNVTTPFKGDDVYLNGDCAFVNVYAGAENGSIISANGDN
LTITGQNHTLSFTDSQGPVLQNYAFISAGETLTLKDFSSLMFSKNVSCGEKGMISGKTVSISGAGEVIFW
DNSVGYSPLSIVPASTPTPPAPAPAPAASSSLSPTVSDARKGSIFSVETSLEISGVKKGVMFDNNAGNFG
TVFRGNSNNNAGSGGSGSATTPSFTVKNCKGKVSFTDNVASCGGGVVYKGTVLFKDNEGGIFFRGNTAYD
DLGILAATSRDQNTETGGGGGVICSPDDSVKFEGNKGSIVFDYNFAKGRGGSILTKEFSLVADDSVVFSN
NTAEKGGGAIYAPTIDISTNGGSILFERNRAAEGGAICVSEASSGSTGNLTLSASDGDIVFSGNMTSDRP
GERSAARILSDGTTVSLNASGLSKLIFYDPVVQNNSAAGASTPSPSSSSMPGAVTINQSGNGSVIFTAES
LTPSEKLQVLNSTSNFPGALTVSGGELVVTEGATLTTGTITATSGRVTLGSGASLSAVAGAANNNYTCTV
SKLGIDLESFLTPNYKTAILGADGTVTVNSGSTLDLVMESEAEVYDNPLFVGSLTIPFVTLSSSSASNGV

TKNSVTINDADAAHYGYQGSWSADWTKPPLAPDAKGMVPPNTNNTLYLTWRPASNYGEYRLDPQRKGE
LVPNSLWVAGSALRTFTNGLKEHYVSRDVGFVASLHALGDYILNYTQDDRDGFLARYGGFQATAASHYE
NGSIFGVAFGQLYGQTKSRMYYSKDAGNMTMLSCFGRSYVDIKGTETVMYWETAYGYSVHRMHTQYFN
DKTQKFDHSKCHWHNNNYYAFVGAEHNFLEYCIPTRQFARDYELTGFMRFEMAGGWSSSTRETGSLTRY
FARGSGHNMSLPIGIVAHAVSHVRRSPPSKLTLNMGYRPDIWRVTPHCNMEIIANGVKTPIQGSPLARHAFF
LEVHDTLYIHHFGRAYMNYSLDARRRQTAHFVSMGLNRIF

SEQ ID NO: 24 (pmpI)

MRPDHMNFCCLCAAILSSTAVLFGQDPLGETALLTKNPNHVVCTFFEDCTMESLFPALCAHASQDDPLYV
LGNSYCWFVSKLHITDPKEALFKEKGDLSIQNFRFLSFTDCSSKESSPSIIHQKNGQLSLRNNGSMSFCR
NHAEGSGGAISADAFSLQHNYLFTAFEENSSKGNGGAIQAQTFSLSRNVSPISFARNRADLNGGAICCSN
LICSGNVNPLFFTGNSATNGGAICCISDLNTSEKGSLSLACNQETLFASNSAKEKGGAIYAKHMVLRYNG
PVSFINNSAKIGGAIAIQSGGSLSILAGEGSVLFQNNSQRTSDQGLVRNAIYLEKDAILSSLEARNGDIL
FFDPIVQESSSKESPLPSSLQASVTSPTPATASPLVIQTSANRSVIFSSERLSEEEKTPDNLTSQLQQPI
ELKSGRLVLKDRAVLSAPSLSQDPQALLIMEAGTSLKTSSDLKLATLSIPLHSLDTEKSVTIHAPNLSIQ
KIFLSNSGDENFYENVELLSKEQNNIPLLTLSKEQSHLHLPDGNLSSHFGYQGDWTFSWKDSDEGHSLIA
NWTPKNYVPHPERQSTLVANTLWNTYSDMQAVQSMINTIAHGGAYLFGTWGSAVSNLFYAHDSSGKPID
NWHHRSLGYLFGISTHSLDDHSFCLAAGQLLGKSSDSFITSTETTSYIATVQAQLATPLMKISAQACYNES
IHELKTKYRSFSKEGFGSWHSVAVSGEVCASIPIVSNGSGLFSSFSIFSKLQGFSGTQDGFEESSGEIRS
FSASSFRNISLPMGITFEKKSQKTRNYYYFLGAYIQDLKRDVESGPVVLLKNAVSWDAPMANLDSRAYMF
RLTNQRALHRLQTLLNVSYVLRGQSHSYSLDLGTTYRF

(3) Major Outer Membrane Protein (MOMP) (CT681)

One example of a MOMP sequence is disclosed as SEQ ID NOS 155 and 156 in International Patent Application No. PCT/IB02/05761 (WO 03/049762). The polypeptide sequence encoding MOMP is set forth below as SEQ ID NO: 25. This protein is thought to function *in vivo* as a porin {ref. xxvi}, and to be present during the whole life cycle of the bacteria {ref. xxvii}. MOMP displays four variable domains (VD) surrounded by five constant regions that are highly conserved among serovars {ref. xxviii xxix}. *In vitro* and *in vivo* neutralizing B-cell epitopes have been mapped on VDs {Ref. xxx, xxxi, xxxii, xxxiii, xxxiv}. T-cell epitopes have been identified in both variable and constant domains {xxxv, xxxvi}.

Preferred MOMP proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 25; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 25, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These MOMP proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 25. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 25, preferably one or more of the B cell or T cell epitopes identified above. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 25. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain). Other preferred fragments include one or more of the conserved constant regions identified above.

SEQ ID NO: 25 (MOMP)(CT681)

MKKLLKSVLVFAALSSASSLQALPVGNPAEPSLMIDGILWEGFGGDPCDPCATWCDAISMRVGYYGDFVF
DRVLKTDVNKEFQMGAKPTTDTGNSAAPSTLTARENPAYGRHMQDAEMFTNAACMALNIWDRFDVFCT
LGATSGYLKGNSASFNLVGLFGDNENQKTVKAESVPNMSFDQSVVELYTDTTFAWSVGARAALWECGCA
TLGASFQYAQSKPKVEELNVLCNAAEFTINKPKGYVGKEFPLDLTAGTDAATGTKDASIDYHEWQASLAL

SYRLNMFTPYIGVKWSRASFDADTIRIAQPKSATAIFDTTTLNPTIAGAGDVKTGAEGQLGDTMQIVSLQLN
KMKSRKSCGIAVGTTIVDADKYAVTVETRLIDERAAHVNAQFRF

(4) Cap1 (CT529)

The *Chlamydia trachomatis* Cap1 protein corresponds with the hypothetical open reading frame CT 529 and refers to Class I Accessible Protein-1. See Ref. xxxvii. One example of a Cap1 protein is set forth herein as SEQ ID NO: 26. Predicted T-cell epitopes of Cap1 are identified in this reference as CSFIGGITYL, preferably SFIGGITYL, and SIIGGITYL.

Preferred Cap1 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 26; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 26, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Cap1 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 26. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 26. Preferred T-cell epitopes include one or more of the T-cell epitopes identified above. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 26. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 26 (Cap1)(CT529)

MASICGRLGSGTGNALKAFFTQPNNKMARVVNKTKGMDKTIKVAKSAAELTANILEQAGGAGSSAHITAS
QVSKGLGDARTVVALGNAFNGALPGTVQSAQSFFSHMKAASQKTQEGDEGLTADLCVSHKRRAAAAVCS
IIGGITYLATFGAIRPILFVNKMLAKPFLSSQTKANMGSSVSYIMAANHAASVVGAGLAISAERADCEARC
ARIAREESLLEVPGEENACEKKVAGEKAKTFTRIKYALLTMLEKFLECVADVFKLVPLPITMGIRAIVAA
GCTFTSAIIGLCTFCARA

(5) GroEL-like hsp60 protein

One example of a *Chlamydia trachomatis* GroEL-like hsp60 protein is set forth herein as SEQ ID NO: 27. The role of Hsp60 in chlamydial infection is further described in, for example, xxxviii, xxxix, xl, xli, and xlii. Immunization of guinea pig models with recombinant Hsp60 is described in xliii. B-cell epitopes of Hsp60 are identified in xliv.

Preferred hsp60 proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 27; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 27, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These hsp60 proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 27. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 27, including one or more of the epitopes identified in the references discussed above. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 27. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an

extracellular domain). Other preferred fragments comprise a polypeptide sequence which does not cross-react with related human proteins.

SEQ ID NO: 27 (groEL-like hsp60 protein)

MVAKNIKYNEEARKKIQKGVKTLAEAVKVTLGPKGRHVVIDKSFGSPQVTKDGVTVAKEVELADKHENM
GAQMVKEVASKTADKAGDGTTTATVLAEAIYTEGLRNVTAGANPMDLKRGIDKAVKVVVDQIRKISKPV
QHHKEIAQVATISANNDAEIGNLIAEAMEKVGKNGSITVEEAKGFETVLDIVEGMNFNRGYLSSYFATNPET
QECVLEDALVLIYDKKISGIKDFLPVLQQVAESGRPLLIIAEDIEGEALATLVVNRIRGGFRVCAVKAPG
FGDRRKAMLEDIAILTGGQLISEELGMKLENANLAMLGKAKKVIVSKEDTTIVEGMGEKEALEARCESIK
KQIEDSSSDYDKEKLQERLAKLSGGVAVIRVGAATEIEMKEKKDRVDDAQHATIAAVEEGILPGGGTALIR
CIPTLEAFLPMLTNEDEQIGARIVLKALSAPLKQIAANAGKEGAIIFQQVMSRSANEGYDALRDAYTDMLEA
GILDPAKVTRSALESAASVAGLLLTTEALIAEIPEEKPAAAPAMPGAGMDY

(6) 60 kDa Cysteine rich protein (OmcB) (CT443)

One example of a *Chlamydia trachomatis* 60kDa Cysteine rich protein is set forth herein as SEQ ID NO: 28. This protein is also generally referred to as OmcB, Omp2 or CT 443. The role of OmcB in chlamydial infection is further described in, for example, xlv, xlvi, xlvii, xlviii, and xlix.

Preferred OmcB proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 28; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 28, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These OmcB proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 28. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 28, including one or more of the epitopes identified in the references discussed above. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 28. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID NO: 28 (omp2/omcB)

MRIGDPMNKLIRRAVTIFAVTSVASLFASGVLETSMAESLSTNVISLADTKAKDNTSHKSKKARKNHSKE
TPVDRKEVAPVHESKATGPKQDSCFGRMYTVKVNDDRNVEITQAVPEYATVGSPYPIEITATGKRDCVDV
IITQQLPCEAEFVRSDPATTPTADGKLVWKIDRLGQGEKSKITVWVKPLKEGCCFTAATVCACPEIRSVT
KCGQPAICVKQEGPENACLRCPVVYKINIVNQGTATARNVVVENPVPDGYAHSSGQRVLTFTLGDMQPGE
HRTITVEFCPLKRGRATNIATVSYCGGHKNTASVTTVINEPCVQVSIAGADWSYVCKPVEYVISVSNPGD
LVLRDVVVEDTLSPGVTVLEAAGAQISCNKVVWTVKELNPGESLQYKVLVRAQTPGQFTNNVVVKSCSD
CGTCTSCAEATTYWKGVAATHMCVVDTCDPVCVGENTVYRICVTNRGSAEDTNVSLMLKFSKELQPVSF
SGPTKGTITGNTVVFDSLPRLGSKETVEFSVTLKAVSAGDARGEAILSSDTLTVPVSDTENTHIY

The immunogenicity of other *Chlamydia trachomatis* antigens of known and unknown biological function may be improved by combination with two or more *Chlamydia trachomatis* antigens from either the first antigen group and/or the second and/or the third antigen group. Such other *Chlamydia trachomatis* antigens of known and unknown biological function include a fourth antigen group consisting of (1) CT559 (YscJ); (2) CT600 (Pal); (3) CT541 (Mip); (4) CT623 (CHLPN 76kDA homologue) (5) CT700 (Hypothetical protein). (6) CT266 (Hypothetical protein); (7) CT077 (Hypothetical protein); (8) CT456 (Hypothetical protein); (9) CT165

(Hypothetical protein) and (10) CT713 (PorB). These antigens are referred to as the "fourth antigen group".

YscJ (CT559)

One example of 'YscJ' protein is disclosed as SEQ ID NO[s]: 199 & 200 in WO 03/049762 {GenBank accession number: AAC68161.1 GI:3329000; 'CT559'; SEQ ID NO: 29 below}. Preferred YscJ proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 29; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 29, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These YscJ proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 29. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 29. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 29. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 29

MFRYTLSRSLFFILALFFCSACDSRSMITHGLSGRDANEIVVLL
VSKGVAAQKVPQAASSTGGSGEQLWDISVPAAQITEALAILNQAGLPRMKGTSLLDLF
AKQGLVPSEMQEKIRYQEGLSEQMATTIRKMDGIVDASVQISFSPEEEDQRPLTASVY
IKHRGVLDNPNSIMVSKIKRLVASAVPGLCPENVSVVSDRASYSDITINGPWGLSDEM
NYVSVWGIILAKHSLTKFRLVFYFLILLLFILSCGLLWVIWKTHTLISALGGTKGFFD
PAPYSQLSFTQNKPAPKETPGAAEGAEAQTASEQPSKENAEKQEENNEDA"

Pal (CT600)

One example of a 'Pal' protein is disclosed as SEQ ID NO[s]: 173 & 174 in WO 03/049762 {GenBank accession number: AAC68202.1 GI:3329044 'CT600'; SEQ ID NO: 30 below}. Preferred Pal proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 30; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 30, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Pal proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 30. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 30. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 30. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 30 (CT600)

MRKTIFKAFNLLFSLLFLSSCSYPCRDWECHGCDSARPRKSSFG
FVPFYSDEEIQQAFVEDFDSKEEQLYKTSAQSTSFRNITFATDSYSIKGEDNLTILAS
LVRHLHKSPKATLYIEGHTDERGAAAYNLALGARRANAVKQYLIKQGIAADRLFTISY
GKEHPVHPGHNELAWQQNRRTEFKIHAR

Mip (CT541)

One example of a 'Mip' protein is disclosed as SEQ ID NO[s]: 149 & 150 in WO 03/049762 {GenBank accession number: AAC68143.1 GI:3328979 'CT541'; SEQ ID NO: 31 below}. Preferred Mip proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 31; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 31, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Mip proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 31. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 31. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 31. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 31 (CT541)

MKNILSWMLMFAVALPIVGCDNGGGSQTSATEKSMVEDSALTDN
QKLSRTFGHLLSRQLSRTEDFSLDLVEVIKGMQSEIDGQSAPLTDTEYEKQMAEVQKA
SFEAKCSENLASAEKFLKENKEKAGVIELEPNKLQYRVVKEGTGRVLSGKPTALLHYT
GSFIDGKVFDSSEKNKEPILLPLTKVIPGFSQGMQGMKEGEVRVLYIHPDLAYGTAGQ
LPPNSLLIFEVKLIEANDDNVSVTE"

CHLPN (76kDa) (CT623)

One example of a CHLPN (76kDa protein) is disclosed as SEQ ID NO[s]: 163 & 164 in WO 03/049762 {GenBank accession number: AAC68227.2 GI:6578109 'CT623'; SEQ ID NO: 32 below}. Preferred CHLPN (76kDa protein proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 32; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 32, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These CHLPN (76kDa protein) proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 32. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 32. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 32. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 32 (CT623)

MKKYFYKGFVGALLLACGSTNLAFAQASSMDSQLWSVEDLDSYL
SSKGFVETRKRDGVLRLAGDVRARWIYAKEDLETTQTPAKPMLPTNRYRSEFNLYVDY
TAANSWMTSKMNWVTIAGGESSAAGLDINRAFLGYRFYKNPETQAEVFAEIGRSGLGD
IFDSDVQFNSNFDGIHLYAARRISEKLPFTMIVHGGPFVVNMAEKEYAWVVEAILNKL
PGNFVVKTSVVDWNTLTAKTNDPADASAAQPAKPNTKYDYLVWQWLVGKSTAMPWFNG
QTKNLYTYGAYLFNPLAEIPENWKQSTTPTTKITNGKENHAWFIGCSLGGVRRAGDWS
ATVRYEYVEALAIPEIDVAGIGRGNQMKYWFAQAIKQGLDPKESNGFTNYKGVSYQFV
MGLTDSVSFRAYAAYSKPANDNLGSDFTYRKYDLGLISSF

Hypothetical Protein (CT700)

One example of a Hypothetical Protein is disclosed as SEQ ID NO[s] 261 & 262 in WO 03/049762 {GenBank accession number: AAC68295.1 GI:3329154 'CT700'; SEQ ID NO: 33

below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 33; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 33, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 33. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 33. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 33. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 33 (CT700)

MWLIVASTLLACLAMALVFKAYRHVISFRSYVNQVIRDVRLSVD
LKEWAVAEMRLAPILKKRQYRRKYLFEYIRILRELERFEEAEKLLGEAKKLKLAGAHF
FLEVAHKAFRHGAYKEAAHAFSLLSAELMGEREVARYTISLVYLGEVDAACRIIEPWI
GPLAHQEVFISVGHIYFATKRYADAIDFYRRARSLGSCPIDVLYNLAHSLRICGQYVD
AGMLFRELLGDPVYKDEAMFNIGLCEQKLGNSKKALLIYQNSELWVRGDALMMRYAAL
AAADQQDYQLAEHCWTLAFRCQSYADDWNCCVHYGLALCHLKKYAEAEKVYLRVIQKT
PDCLVACKALAWLAGVGHATMISAREGIAYAKRALQIKRSPEVLELLSACEAREGNFD
VAYDIQAILAERDTTAKERERRSQILKNLRQKLPIDQQHIVEVSLLLAA

Hypothetical Protein (CT 266)

One example of a Hypothetical Protein is disclosed as SEQ ID NO[s] 77 & 78 in WO 03/049762 {GenBank accession number: AAC67859.1 GI:3328678 'CT266'; SEQ ID NO: 34 below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 34; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 34, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 34. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 34. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 34. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 34 (CT266)

MLVESQLGLEDVLEAFSERNFDIQSKSFIESFQDKKLRRTVIQR
FLHHPLLHIHDIARAAYLLAALEEGVDLGYQFLCMHQTQSGAALLFRRAGFLWGGLPY
PGEHAEMAMLLSRIAEFYDTSYEQVQKMIAFQHALFSHERNIFPALWSQEGSRSNQEK
TAVSKLLFCQKEARIEDQFTLTDMSLGFWMRRTPSFSAYVSGSGCKSGVGAFLIGDVG
VLNYGPCVGDPGECLGFGLCGQVKEFSCQEKDEEVSISFAGALSQPSSRRTGFSYLQD
ALFSTNSCYCIDITEQKCHVASSLDRENQDAFFAIFCKGSQCQVCNGPKLRTGSPDSY
KGPAYDVLIKGEKETVRILSSSPHMEIFSLQGKDRFWGSNFLINLPYTQNSINILFEKA

Hypothetical Protein (CT077)

One example of a Hypothetical Protein is disclosed as SEQ ID NO[s] 65 & 66 in WO 03/049762 {GenBank accession number: AAC67668.1 GI:3328472 'CT077'; SEQ ID NO: 35 below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a)

having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 35; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 35, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 35. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 35. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 35. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 35 (CT077)

MGKFFASYLLILAPFFLQSCSAPSRTTLEGVRMTIPYRIVFGEA
LSPDAFQQAQKEIDRVFDHIDQTFNNWNPLSEISRINRTTKQTPIPLSPALFAFLCEI
DHFHAFSDGRFDPTLGALKSLWLLHLKSHTIPSQELQHLYKHSSGWHLISLDKTQQTL
RKLSPLVQLDLCGTVKGFAVDLLGTACAQFCQNYYVEWGGEIKTKGKHPSGRSWAVAS
SATPEILHLHDHAIATSGSQYQRWHVDNKTYTHILDPLTGTPLEDSSHPILAVSVINE
SCAFADAMATALTTFSSKQEALDWANKKHLCAYITDKNVS

Hypothetical Protein (CT456)

One example of a Hypothetical Protein is disclosed as SEQ ID NO[s] 255 & 256 in WO 03/049762 {GenBank accession number: AAC68056.1 GI:3328889 'CT456'; SEQ ID NO: 36 below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 36; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 36, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 36. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 36. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 36. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 36 (CT456)

MTNSISGYQPTVTTSTSSTTSASGASGSLGASSVSTTANATVTQ
TANATNSAATSSIQTTGETVVNYTNSASAPNVTVSTSSSSTQATATSNKTSQAVAGKI
TSPDTSESSETSSTSSSDHIPSDYDDVGSNSGDISNNYDDVGSNNGDISSNYDDAAAD
YEPIRTTENIYESIGGSRTSGPENTSGGAAAALNSLRGSSYSNYDDAAADYEPIRTTE
NIYESIGGSRTSGPENTSGGAAAALNSLRGSSYSNYDDAAADYEPIRTTENIYESIGG
SRTSGPENTSDGAAAAALNSLRGSSYTTGPRNEGVFGPGPEGLPDMSLPSYDPTNKTS
LLTFLSNPHVKSKMLENSGHFVFIDTDRSSFILVPNGNWDQVCSIKVQNGKTKEDLDI
KDLENMCAKFCTGFSKFSGDWDSLVEPMVSAKAGVASGGNLPNTVIINNKFKTCVAYG
PWNSQEASSGYTPSAWRRGHRVDFGGIFEKANDFNKINWGTQAGPSSEDDGISFSNET
PGAGPAAAPSPTPSSIPIINVNVNVGGTNVNIGDTNVNTTNTTPTTQSTDASTDTSDI
DDINTNNQTDDINTTDKDSDGAGGVNGDISETESSSGDDSGSVSSSESDKNASVGNDG
PAMKDILSAVRKHLDVVYPGENGGSTEGPLPANQTLGDVISDVENKGSAQDTKLSGNT
GAGDDDPTTTAAVGNGAEEITLSDTDSGIGDDVSDTASSSGDESGGVSSPSSESNKNT
AVGNDGPSGLDILAAVRKHLDKVYPGDNGGSTEGPLQANQTLGDIVQDMETTGTSQET
VVSPWKGSTSSTESAGGSGSVQTLLPSPPPTPSTTTLRTGTGATTTSLMMGGPIKADI
ITTGGGGRIPGGGTLEKLLPRIRAHLDISFDAQGDLVSTEEPQLGSIVNKFRQETGSR

GILAFVESAPGKPGSAQVLTGTGGDKGNLFQAAAAVTQALGNVAGKVNLAIQGGQKLSS
LVNDDGKGSVGRDLFQAAAQTTQVLSALIDTVG"

Hypothetical Protein (CT165)

One example of a Hypothetical Protein is disclosed {GenBank accession number: AAC67756.1 GI:3328568 CT165'; SEQ ID NO: 37 below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 36; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 37, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 37. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 37. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 37. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 37 (CT165)

MFQPETVPSNRSTETTPQNIEVYNDRNFTNHTTEDVIRIGERLQ
RQFYNMTEESRVPFTTSPSHHTGNWKTAFLYNLSQVVAHIFPSTVQPIRVKPTRIPPS
PTPPPEGTTTAETSTSENKVTTISKEQEVTTKPLLVRERRSLLHSQ

PorB (CT713)

One example of a PorB Protein is disclosed as SEQ ID NO[s] 201 & 202 in WO 03/049762 {GenBank accession number: AAC68308.1 GI:3329169 'CT713'; SEQ ID NO: 38 below}. Preferred PorB proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 38; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 38, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These PorB proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 38. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 38. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 38. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 38 (CT713)

MSSKLVNYLRLTFLSFLGIASTSLDAMPAGNPAFPVIPGINIEQ
KNACSFDLCNSYDVLSALSGNLKLCFCGDYIFSEEAQVKDVPVVTSVTTAGVGPSPDI
TSTTKTRNFDLVNCNLNTNCVAVAFSLPDRSLSAIPLFDVSFEVKVGGLKQYYRLPMN
AYRDFTSEPLNSESEVTDGMIEVQSNYGFVWDVSLKKVIWKDGVSFVGVGADYRHASC
PIDYIIANSQANPEVFIADSDGKLNFKEWSVCVGLTTYVNDYVLPYLAFSIGSVSRQA
PDDSFKKLEDRFTNLKFKVRKITSSHRGNICIGATNYVADNFFYNVEGRWGSQRAVNV
SGGFQF

The immunogenicity of other Chlamydia trachomatis antigens of known and unknown biological function may be improved by combination with two or more *Chlamydia trachomatis* antigens from either the first antigen group and/or the second and/or the third antigen group and//or the

fourth antigen group. Such other *Chlamydia trachomatis* antigens of known and unknown biological function include a fifth antigen group consisting of: (1) CT082 (hypothetical); (2) CT181 (Hypothetical); (3) CT050 (Hypothetical); (4) CT157 (Phospholipase D superfamily); and (5) CT128 (AdK adenylate cyclase).

Hypothetical Protein (CT082)

One example of a Hypothetical Protein is disclosed as {GenBank accession number: AAC67673.1 GI:3328477 'CT082'; SEQ ID NO: 39 below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 39; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 39, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 39. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 39. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 39. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 39 (CT082)

MSISGSGNVSPATPDFDPSILMGRQAASAHAAKEASGASKATET
SAAEQQALISSGTELDYVTDLQQSEGKYKKTLDKTSKSPKTKLKGNFSKVRAGTKGFL
TGFGTRASRISARKAENNGEGMSMIPSQMEYVKKKGNRVSPEMQNFYLGASGLWSPTS
DVSSITENCLGATALSTTPLLTTMQDPVSIEHLSSGEITALASFNPNVRTASLNEQTI
NAWTEARLGGEMVSTLLDPNIETSSLLRRAPTVSNEGMVDVSDMGNQTTSLSMEGLVN
TVVDDPASAEEEKKTGELSLEEMAAMAKMMAALLSSGQGMAVFIASSTPSSGLTQFPE
PKFSGTIPHHFSKKEDNETIWGLDSQIGSIAFDTRRENNASPLPTTSLHEEASYRFPV
GEAPLDVNEIPFAVQHSTVFSKETANTEQALIQNESLGEIPVSAEVVGQDTVSSAYQF
PSHLGMAVLASVPLSTEDYKTAVEHRKGPGGPPDPLIYQYRNVAVDPAIIFQSPSPFS
VSSRFSVQGKPEAVAVYNDDQEEAAGGNRDSDEGKDQEQDKTRETEDAGGDS

Hypothetical Protein (CT181)

One example of a Hypothetical Protein is disclosed as SEQ ID NO[s] 245 & 246 in WO 03/049762 {GenBank accession number: AAC67772.1 GI:3328585 'CT181'; SEQ ID NO: 40 below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 40; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 40, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 40. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 40. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 40. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 40 (CT181)

MLSKFCKLSLSAILLINTLAPSETFSEEGTSGFLGRMKSWILKD
KTILSTTEESQTSAIEKVSDLLSWKRYDYTQESGFAIQFPESPEHSEQVIEVPQSDLA

IRYDTYVAETPSDSTVYVVSIWEYPEKIDISRPELNLQEGFAGMLYALPESQVLYLKA
TALQGHKALEFWIACDDVYFRGMLVSVNHTLYQVFMVYKGRSPEILDKEYSTFIQSFK
VTKVRNSKKMDIRKRVSL

Hypothetical Protein (CT050)

One example of a Hypothetical Protein is disclosed as {GenBank accession number: AAC67641.1 GI:3328442 'CT050'; SEQ ID NO: 41 below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 40; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 41, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 41. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 41. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 41. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 41 (CT050)

MNDTKNNISSSFWNPNKVVTKVLLKVSETGIESTPGIVKHNQLI
TQSENPTDPTDAVTFKYLKENYTKENDPNPGFLPTTGGTMTGDIDMQGNNVTDIVMYT
NGQQNPTDDSAVTIGYLNEKADEIKSNDQITTAVAGLSNINSQISTLHQLLGIAEDPD
TVTNPDLLKTSGGTVYEDIDMSSNTVSDLGTPTNKDTKSAINVEFVQAKITSPQMAFL
KNNDTNLSNITVSEYFNWLQDPTQAPTPEPDPDPEPAPEPEPDTSDSSGSGSENPADP
APTNPSDSNAQNNPTPSSNGATASIRKLAATTTTVPTDTEIAPAAEDPNLPNTTFSEK
SPLWEEFFSFSDSSRSEMVIQKTGILTFSMQGTWENPSSSQTPSTDPISLELTVTPPT
TDTPPESPPSPPEAPAPEATPSPTNNNLTASITKTFSRKYNLSATPSPTPTTPTEPTT
ITKTLSLSSGQSCTLQIPVQATRSVLKLKYVNPNNNSSGGSSGSGGSSQPETTPTGIT
LQSFSWSLVLTPGEITKATSTPSTPSQP

Phospholipase D SuperFamily (CT157)

One example of a Phospholipase D SuperFamily Protein is disclosed as {GenBank accession number: AAC67748.1 GI:3328559 'CT157'; SEQ ID NO: 42 below}. Preferred Phospholipase D SuperFamily proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 42; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 42, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Phospholipase D SuperFamily proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 42. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 42. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 42. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 42 (CT157)

MSVQGSSSLKYSDLFKPPEPTSSTDSSKEPPKESAWKVVSHSRG
RRRARSNPSPHTSQNTPSPKDSSLVARTDKAATDIFNSAKHKAIETTKRSDQQSRSLH
ILHLLAENPEPIVFHSAHQTNHNDPQRMLCDAILQANRIITMRIFNIGSPEIIRALIR

AVRRNIPVVVSAWNFPNLSNWDRESELCVELRGNPQICLHKKTTLIDNQLTIIGTANY
TKSSFFKDINLTALIQNPALYSLILSDTRGSVSIGSQTISYYPLPFPQSNTKILPIIQ
EIQKAQRTIKIAMNIFSHTEIFLALEQARLRGVTITIVINKKESAHTLDILHRISALL
LLKSVTTVDSLHAKICLIDNQTLIFGSPNWTYHGMHKNLEDLLIVTPLTPKQIHSIQE
IWAFLLKNSSPV

AdK (Adenylate Kinase) (CT128)

One example of an Adenylate Kinase Protein is disclosed as {GenBank accession number: AAC67719.1 GI:3328527 'CT128'; SEQ ID NO: 43 below}. Preferred Adenylate Kinase proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 43; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 43, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Adenylate Kinase proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 43. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 43. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 43. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 43 (CT128)

MDRSPLFLIIMGAPGSGKGTQSKLLASQLSLLHISSGDLLRDAV
SKDTPLSQEIKSYLDQGKLLPDTLVWKLVHEKLDEFQQDTLLRRLSFLSRSENSAILD
GFPRTVTQAKLLHEFLSSYFPNYKVILLDISDEEVLNRLTSRYICPACQGIYNEQQGF
SSCPKCSVELIRRSDDTLEVILDRIQTYKQETQPVLDYYTEKQKLITIDANAPTQQVF
QSILDSLSASLVYQERDCCNCDCDDED

The immunogenicity of other Chlamydia trachomatis antigens of known and unknown biological function may be improved by combination with two or more *Chlamydia trachomatis* antigens from either the first antigen group and/or the second and/or the third antigen group and//or the fourth antigen group and/or the Such other *Chlamydia trachomatis* antigens of unknown biological function include a sixth antigen group consisting of: (1) CT153 (Hypothetical); (2) CT262 (Hypothetical); (3) CT276 (Hypothetical); (4) CT296 (Hypothetical); (5) CT372 (Hypothetical); (6) CT412 (PmpA); (7) CT480 (OligoPeptide Binding Protein); (8) CT548 (Hypothetical); (9) CT043 (Hypothetical); (10) CT635 (Hypothetical); (11) CT859 (Metalloprotease);(12) CT671 (Hypothetical); (13) CT016 (Hypothetical); (14) CT017 (Hypothetical); (15) CT043 (Hypothetical); (16) CT082 (Hypothetical); (17)CT548 (Hypothetical); (19) CT089 (Low Calcium Response Element); (20) CT812 (PmpD) and (21) CT869 (PmpE).

Hypothetical Protein (CT153)

One example of a Hypothetical Protein is disclosed as {GenBank accession number: AAC67744.1 GI:3328555 'CT153'; SEQ ID NO: 44 below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 44; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 44, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 44. Preferred

fragments of (b) comprise an epitope from SEQ ID NO: 44. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 44. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 44 (CT153)

MTKPSFLYVIQPFSVFNPRLGRFSTDSDTYIEEENRLASFIESL
PLEIFDIPSFMETAISNSPYILSWETTKDGALFTILEPKLSACAATCLVAPSIQMKSD
AELLEEIKQALLRSSHDGVKYRITRESFSPEKKTPKVALVDDDIELIRNVDFLGRAVD
IVKLDPINILNTVSEENILDYSFTRETAQLSADGRFGIPPGTKLFPKPSFDVEISTSI
FEETTSFTRSFSASVTFSVPDLAATMPLQSPPMVENGQKEICVIQKHLFPSYSPKLVD
IVKRYKREAKILINKLAFGMLWRHRAKSQILTEGSVRLDLQGFTESKYNYQIQVGSHT
IAAVLIDMDISKIQSKSEQAYAIRKIKSGFQRSLDDYHIYQIERKQTFSFSPKHRSLS
STSHSEDSDLDLSEAAAFSGSLTCEFVKKSTQHAKNTVTCSTAAHSLYTLKEDDSSNP
SEKRLDSCFRNWIENKLSANSPDSWSAFIQKFGTHYIASATFGGIGFQVLKLSFEQVE
DLHSKKISLETAAANSLLKGSVSSSTESGYSSYSSTSSSHTVFLGGTVLPSVHDERLD
FKDWSESVHLEPVPIQVSLQPITNLLVPLHFPNIGAAELSNKRESLQQAIRVYLKEHK
VDEQGERTTFTSGIDNPSSWFTLEAAHSPLIVSTPYIASWSTLPYLFPTLRERSSATP
IVFYFCVDNNEHASQKILNQSYCFLGSLPIRQKIFGSEFASFPYLSFYGNAKEAYFDN
TYYPTRCGWIVEKLNTTQDQFLRDGDEVRLKHVSSGKYLATTPLKDTHGTLTRTTNCE
DAIFIIKKSSGY"

Hypothetical Protein (CT262)

One example of a Hypothetical Protein is disclosed as {GenBank accession number: AAC67835.1 GI:3328652'CT262'; SEQ ID NO: 45 below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 45; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 45, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 45. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 45. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 45. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 45 (CT242)

MKKFLLLSLMSLSSLPTFAANSTGTIGIVNLRRCLEESALGKKE
SAEFEKMKNQFSNSMGKMEEELSSIYSKLQDDDYMEGLSETAAAELRKKFEDLSAEYN
TAQGQYYQILNQSNLKRMQKIMEEVKKASETVRIQEGLSVLLNEDIVLSIDSSADKTD
AVIKVLDDSFQNN

Hypothetical Protein (CT276)

One example of a Hypothetical Protein is disclosed as {GenBank accession number: AAC67869.1 GI:3328689 'CT276'; SEQ ID NO: 46 below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 46; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 46, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35,

40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 46. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 46. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 46. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide

SEQ ID NO 46 (CT276)

MFKRPAKNFFDEVQTLYEDSGANSTSYSIYPQRTERLENHSNIF
EPAKPAETRLLSQEEHSQWTDQQEELATQESSFPEEPETTLGEGVSFKGELTFERLLR
IDGTFEGILVSKGKIIVGPQGYVKANIELEEAVIAGVVEGNITVTGRVSLQGRAMVTG
DIQAGSLCVDEGVRLCGYVSIQGAPSNEQEEIDS

Hypothetical Protein (CT296)

One example of a Hypothetical Protein is disclosed as {GenBank accession number: AAC67889.1 GI:3328711 'CT296'; SEQ ID NO: 47 below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 47; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 47, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 47. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 47. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 47. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide.

SEQ ID No 47 (CT296)

MRAVLHLEHKRYFQNHGHILFEGLAPVSDCKQLEAELKLFLKEV
AVVKDRHLQRWRENVHRTLPEVQMIVKRVRLDHLAAELTHRSRVALVRDLWVQKQEEI
FFDDCDCSVLLCLSGEKAGWGLFFSGEYPQDVFNWGAGDTAIILRFSSAGFPN

Hypothetical Protein (CT372)

One example of a Hypothetical Protein is disclosed as SEQ ID NO[s] 187 & 188 in WO 03/049762 {GenBank accession number: AAC67968.1 GI:3328796 'CT372'; SEQ ID NO: 48 below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 48; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 48, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 48. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 48. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 48. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 48 (CT372)

MQAAHHHYHRYTDKLHRQNHKKDLISPKPTEQEACNTSSLSKEL
IPLSEQRGLLSPICDFISERPCLHGVSVRNLKQALKNSAGTQIALDWSILPQWFNPRV
SHAPKLSIRDFGYSAHQTVTEATPPCWQNCFNPSAAVTIYDSSYGKGVFQISYTLVRY
WRENAATAGDAMMLAGSINDYPSRQNIFSQFTFSQNFPNERVSLTIGQYSLYAIDGTL
YNNDQQLGFISYALSQNPTATYSSGSLGAYLQVAPTASTSLQIGFQDAYNISGSSIKW
SNLTKNRYNFHGFASWAPRCCLGSGQYSVLLYVTRQVPEQMEQTMGWSVNASQHISSK
LYVFGRYSGVTGHVFPINRTYSFGMASANLFNRNPQDLFGIACAFNNVHLSASPNTKR
KYETVIEGFATIGCGPYLSFAPDFQLYLYPALRPNKQSARVYSVRANLAI

Putative Outer Membrane Protein A (PmpA) (CT412)

One example of a PmpA Protein is disclosed as SEQ ID NO[s] 89 & 90 in WO 03/049762 {GenBank accession number: AAC68009.1 GI:3328840 'CT412'; SEQ ID NO: 49 below}. Preferred PmpA proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 49; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 49, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These PmpA proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 49. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 49. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 49. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 49 (CT412)

MNRVIEIHAHYDQRQLSQSPNTNFLVHHPYLTLIPKFLLGALIV
YAPYSFAEMELAISGHKQGKDRDTFTMISSCPEGTNYIINRKLILSDFSLLNKVSSGG
AFRNLAGKISFLGKNSSASIHFKHININGFGAGVFSESSIEFTDLRKLVAFGSESTGG
IFTAKEDISFKNNHHIAFRNNITKGNGGVIQLQGDMKGSVSFVDQRGAIIFTNNQAVT
SSSMKHSGRGGAISGDFAGSRILFLNNQQITFEGNSAVHGGAIYNKNGLVEFLGNAGP
LAFKENTTIANGGAIYTSNFKANQQTSPILFSQNHANKKGGAIYAQYVNLEQNQDTIR
FEKNTAKEGGGAITSSQCSITAHNTIIFSDNAAGDLGGGAILLEGKKPSLTLIAHSGN
IAFSGNTMLHITKKASLDRHNSILIKEAPYKIQLAANKNHSIHFFDPVMALSASSSPI
QINAPEYETPFFSPKGMIVFSGANLLDDAREDVANRTSIFNQPVHLYNGTLSIENGAH
LIVQSFKQTGGRISLSPGSSLALYTMNSFFHGNISSKEPLEINGLSFGVDISPSNLQA
EIRAGNAPLRLSGSPSIHDPEGLFYENRDTAASPYQMEILLTSDKIVDISKFTTDSLV
TNKQSGFQGAWHFSWQPNTINNTKQKILRASWLPTGEYVLESNRVGRAVPNSLWSTFL
LLQTASHNLGDHLCNNRSLIPTSYFGVLIGGTGAEMSTHSSEEESFISRLGATGTSII
RLTPSLTLSGGGSHMFGDSFVADLPEHITSEGIVQNVGLTHVWGPLTVNSTLCAALDH
NAMVRICSKKDHTYGKWDTFGMRGTLGASYTFLEYDQTMRVFSFANIEATNILQRAFT
ETGYNPRSFSKTKLLNIAIPIGIGYEFCLGNSSFALLGKGSIGYSRDIKRENPSTLAH
LAMNDFAWTTNGCSVPTSAHTLANQLILRYKACSLYITAYTINREGKNLSNSLSCGGY
VGF

Oligopeptide Binding Lipoprotein (CT480)

One example of an OligoPeptide Binding Protein is disclosed as SEQ ID NO[s] 141 & 142 in WO 03/049762 {GenBank accession number: AAC68080.1 GI:3328915 'CT480'; SEQ ID NO: 50 below}. Preferred OligoPeptide Binding proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 50; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 50, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or

more). These OligoPeptide Binding proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 50. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 50. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 50. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 50 (CT480)

MIDKIIRTILVLSLFLLYWSSDLLEKDVKSIKRELKALHEDVLE
LVRISHQQKNWVQSTDFSVSPEISVLKDCGDPAFPNLLCEDPYVEKVVPSLLKEGFVP
KGILRTAQVGRPDNLSPFNGFVNIVRFYELCVPNLAVEHVGKYEEFAPSLALKIEEHY
VEDGSGDKEFHIYLRPNMFWEPIDPTLFPKNITLADSFLRPHPVTAHDVKFYYDVVMN
PYVAEMRAVAMRSYFEDMVSVRVENDLKLIVRWRAHTVRNEQGEEEKKVLYSAFANTL
ALQPLPCFVYQHFANGEKIVPEDSDPDTYRKDSVWAQNFSSHWAYNYIVSCGAFRFAG
MDDEKITLVRNPNYHNPFAALVEKRYIYMKDSTDSLFQDFKAGKVDIAYFPPNHVDNL
ASFMQTSAYKEQAARGEAILEKNSSDRSYSYIGWNCLSLFFNNRSVRQAMNMLIDRDR
IIEQCLDGRGVSVSGPFSLCSPSYNRDVEGWQYSPEEAARKLEEEGWIDADGDGIREK
VIDGVVVPFRFRLCYYVKSVTARTIAEYVATVCKEVGIECCLLGLDMADYSQALEEKN
FDAILSGWCLGTPPEDPRALWHSEGALEKGSANAVGFCNEEADRIIEQLSYEYDSNKR
QALYHRFHEVIHEESPYAFLYSRQYSLVYKEFVKNIFVPTEHQDLIPGAQDETVNLSM
LWVDKEEGRCSAIS

Hypothetical Protein (CT548)

One example of a Hypothetical Protein is disclosed as SEQ ID NO[s] 153 & 154 in WO 03/049762 {GenBank accession number: AAC68150.1 GI:3328987 'CT548'; SEQ ID NO: 51 below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 51; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 51, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 51. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 51. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 51. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 51 (CT548)

MLKMFWLNSLVFFSLLLSACGYTVLSPHYVEKKFSLSEGIYVCP
IEGDSLGDLVSSLSYELEKRGLHTRSQGTSSGYVLKVSLFNETDENIGFAYTPQKPDE
KPVKHFIVSNEGRLALSAKVQLIKNRTQEILVEKCLRKSVTFDFQPDLGTANAHQLAL
GQFEMHNEAIKSASRILYSQLAETIVQQVYYDLF

Hypothetical Protein (CT043)

One example of a Hypothetical Protein is disclosed as {GenBank accession number: AAC67634.1 GI:3328435 'CT043'; SEQ ID NO: 52 below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 52; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 52, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35,

40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 52. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 52. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 52. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 52 (CT043)

MSRQNAEENLKNFAKELKLPDVAFDQNNTCILFVDGEFSLHLTY
EEHSDRLYVYAPLLDGLPDNPQRRLALYEKLLEGSMLGGQMAGGGVGVATKEQLILMH
CVLDMKYAETNLLKAFAQLFIETVVKWRTVCSDISAGREPTVDTMPQMPQGGGGGIQP
PPAGIRA

Hypothetical Protein (CT635)

One example of a Hypothetical Protein is disclosed as {GenBank accession number: AAC68239.1 GI:3329083 'CT635'; SEQ ID NO: 53 below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 53; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 53, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 53. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 53. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 53. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 53 (CT635)

MKNNSAQKIIDSIKQILSIYKIDFEPSFGATLTDDNDLDYQMLI
EKTQEKIQELDKRSQEILQQTGMTREQMEVFANNPDNFSPEEWRALENIRSSCNEYKK
ETEELIKEVTNDIGHSSHKSPTPKKTKSSSQKKSKKKNWIPL

Metalloprotease ***(CT859)***

One example of a Metalloproease Protein is disclosed as {GenBank accession number: 'CT859' AAC68457.1 GI:3329333; SEQ ID NO: 54 below}. Preferred Metalloprotease proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (*e.g.* 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 54; and/or (b) which is a fragment of at least *n* consecutive amino acids of SEQ ID NO: 54, wherein *n* is 7 or more (*e.g.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Metalloprotease proteins include variants (*e.g.* allelic variants, homologs, orthologs, paralogs, mutants, *etc.*) of SEQ ID NO: 54. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 54. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 54. Other fragments omit one or more domains of the protein (*e.g.* omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 54 (CT859)

MRKIILCSPRGFCAGVIRAIQTVEVALEKWGRPIYVKHEIVHNR
HVVDKLREKGAIFIEDLQEVPRNSRVIFSAHGVPPSVREEAEERGLIAIDATCGLVTK
VHSAVKMYAKKGYHIILIGKRKHVEIIGIRGEAPDQITVVENIAEVEALPFSAQDPLF
YVTQTTLSMDDAADIVAALKARYPRIFTLPSSSICYATQNRQGALRNILPQVDFVYVI
GDTQSSNSNRLREVAERRGVTARLVNHPDEVTEEILQYSGNIGITAGASTPEDVVQAC
LMKLQELIPDLSIEMDLFVEEDTVFQLPKEL

Hypothetical Protein (CT671)

One example of a Hypothetical Protein is disclosed as {GenBank accession number: AAC68266.1 GI:3329122 'CT671'; SEQ ID NO: 55 below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 55; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 55, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 55. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 55. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 55. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 55 (CT671)

MELNKTSESLFSAKIDHNHPRTEAHEPRDQREVRVFSLEGRSST
RQEKADRMPGRTSSRQESSKGSEEGAVHESTAGVSSKEEEESKGDGFFTGGNPTSGMA
LVETPMAVVSEAMVETSTMTVSQVDLQWVEQLVTSTVESLLVADIDGKQLVEIVLDNS
NTVPAAFCGANLTLVQTGEEISVSFSNFVDQAQLTEATQLVQQNPKQLVSLVESLKAR
QLNLTELVVGNVAVSLPTIEKIETPLHMIAATIRHHDQEGDQEGEGRQDQHQGQHQEK
KVEEAHI

Hypothetical Protein (CT016)

One example of a Hypothetical Protein is disclosed as {GenBank accession number: AAC67606.1 GI:3328405 'CT016'; SEQ ID NO: 56 below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 56; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 56, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 56. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 56. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 56. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 56 (CT016)

MKVKINDQFICISPYISARWNQIAFIESCDGGTEGGITLKLHLI
DGETVSIPNLGQAIVDEVFQEHLLYLESTAPQKNKEEEKISSLLGAVQQMAKGCEVQV

FSQKGLVSMLLGGAGSINVLLQHSPEHKDHPDLPTDLLERIAQMMRSLSIGPTSILAK
PEPHCNCLHCQIGRATVEEEDAGVSDEDLTFRSWDISQSGEKMYTVTDPLNPEEQFNV
YLGTPIGCTCGQPYCEHVKAVLYT"

Hypothetical Protein (CT017)

One example of a Hypothetical Protein is disclosed as {GenBank accession number: AAC67607.1 GI:3328406 'CT017'; SEQ ID NO: 57 below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 57; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 57, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 57. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 57. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 57. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 57 (CT017)

MLIFALSFGADACLCAADLSKAKVEASVGDRAAFSPFTGEIKGN
RVRLRLAPHTDSFIIKELSKGDCLAVLGESKDYYVVAAPEGVRGYVFRTFVLDNVIEG
EKVNVRLEPSTSAPILARLSKGTVVKTLGAAQGKWIEIALPKQCVFYVAKNFVKNVGA
LDLYNQKEGQKKLALDLLSSAMDFADAELQKKIEDIDLDAIYKKMNLAQSEEFKDVPG
LQSLVQKALERVQEAFLAKSLEKSSVKVPEIRHKVLEEIAVVSPAVEETPVVTKTEEQ
KVTTVPVPAPAVVTEPAQDLSSVKGSLLSHYIRKKGFVKASPVIEGRESFERSLFAVW
LSLQPEEIRHQLTMESFYRDEQKKKRVLTGELEVYPHIVKNNPGDYLLKNGEDVVAFV
YATSIDLSKWLGKSVVLECVSRPNNHFAFPAYIVLSVKEGA

Hypothetical Protein (CT043)

One example of a Hypothetical Protein is disclosed as {GenBank accession number: AAC67634.1 GI:3328435 'CT043'; SEQ ID NO: 58 below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 58; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 58, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 58. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 58. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 58. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 58 (CT043)

MSRQNAEENLKNFAKELKLPDVAFDQNNTCILFVDGEFSLHLTY
EEHSDRLYVYAPLLDGLPDNPQRRLALYEKLLEGSMLGGQMAGGGVGVATKEQLILMH
CVLDMKYAETNLLKAFAQLFIETVVKWRTVCSDISAGREPTVDTMPQMPQGGGGGIQP
PPAGIRA

SEQ ID No 39 (CT082) – Hypothetical is already discussed above as SEQ ID No 39

Hypothetical Protein (CT548)

One example of a Hypothetical Protein is disclosed as {GenBank accession number: AAC68150.1 GI:3328987 'CT548'; SEQ ID NO: 59 below}. Preferred Hypothetical proteins for use with the invention comprise an amino acid sequence: (a) having 50% or more identity (e.g. 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more) to SEQ ID NO: 59; and/or (b) which is a fragment of at least n consecutive amino acids of SEQ ID NO: 58, wherein n is 7 or more (e.g. 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more). These Hypothetical proteins include variants (e.g. allelic variants, homologs, orthologs, paralogs, mutants, etc.) of SEQ ID NO: 58. Preferred fragments of (b) comprise an epitope from SEQ ID NO: 59. Other preferred fragments lack one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the C-terminus and/or one or more amino acids (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 or more) from the N-terminus of SEQ ID NO: 59. Other fragments omit one or more domains of the protein (e.g. omission of a signal peptide, of a cytoplasmic domain, of a transmembrane domain, or of an extracellular domain).

SEQ ID No 59(CT548)

MLKMFWLNSLVFFSLLLSACGYTVLSPHYVEKKFSLSEGIYVCP
IEGDSLGDLVSSLSYELEKRGLHTRSQGTSSGYVLKVSLFNETDENIGFAYTPQKPDE
KPVKHFIVSNEGRLALSAKVQLIKNRTQEILVEKCLRKSVTFDFQPDLGTANAHQLAL
GQFEMHNEAIKSASRILYSQLAETIVQQVYYDLF

SEQ ID No 3 (CT089) – Low Calcium Response Element (LcrE) - already discussed above
SEQ ID No 19 (CT812) – PmpD – already discussed above
SEQ ID No 20 (CT869) – PmpE – already discussed above

The invention thus includes a composition comprising a combination of *Chlamydia trachomatis* antigens, said combination selected from the group consisting of two, three, four, or five *Chlamydia trachomatis* antigens of the first antigen group and one, two, three, four, five or six *Chlamydia trachomatis* antigens of the third antigen group and one, two, three, four, five, six, seven, eight, nine or ten antigens of the fourth antigen group and one, two, three, four, five or six *Chlamydia trachomatis* antigens of the fifth antigen group and one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve antigens of the sixth antigen group

Preferably, the combination is selected from the group consisting of three, four, or five *Chlamydia trachomatis* antigens from the first antigen group and three, four, or five *Chlamydia trachomatis* antigens from the third antigen group and three, four or five *Chlamydia trachomatis* antigens from the fourth antigen group and one, two, three, four, five or six *Chlamydia trachomatis* antigens of the fifth antigen group and one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve antigens of the sixth antigen group

Still more preferably, the combination consists of five *Chlamydia trachomatis* antigens from the first antigen group and three, four or five *Chlamydia trachomatis* antigens from the third antigen group and three, four or five antigens from the fourth antigen group and one, two, three, four, five or six *Chlamydia trachomatis* antigens of the fifth antigen group and one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve antigens of the sixth antigen group

The invention further includes a composition comprising a combination of *Chlamydia trachomatis* antigens, said combination selected from the group consisting of two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or thirteen *Chlamydia trachomatis* antigens of the second antigen group and one, two, three, four, five or six *Chlamydia trachomatis* antigens of the third antigen group and one, two, three, four, five, six, seven, eight or nine antigens of the fourth antigen group. Preferably, the combination is selected from the group consisting of three, four, or five *Chlamydia trachomatis* antigens from the second antigen group and three, four or five *Chlamydia trachomatis* from the third antigen group and three, four or five antigens of the fourth antigen group. Still more preferably, the combination consists of five *Chlamydia trachomatis* antigens from the second antigen group and three, four or five *Chlamydia trachomatis* antigens of the third antigen group and three, four or five antigens of the fourth antigen group.

There is an upper limit to the number of *Chlamydia trachomatis* antigens which will be in the compositions of the invention. Preferably, the number of *Chlamydia trachomatis* antigens in a composition of the invention is less than 20, less than 19, less than 18, less than 17, less than 16, less than 15, less than 14, less than 13, less than 12, less than 11, less than 10, less than 9, less than 8, less than 7, less than 6, less than 5, less than 4, or less than 3. Still more preferably, the number of *Chlamydia trachomatis* antigens in a composition of the invention is less than 6, less than 5, or less than 4.The *Chlamydia trachomatis* antigens used in the invention are preferably isolated, i.e., separate and discrete, from the whole organism with which the molecule is found in nature or, when the polynucleotide or polypeptide is not found in nature, is sufficiently free of other biological macromolecules so that the polynucleotide or polypeptide can be used for its intended purpose.

Preferably, the composition of the present invention comprises a combination of *Chlamydia trachomatis* antigens, said combination selected from the group consisting of:
(1) CT016 and CT128 and CT671 and CT262; (2) CT296 and CT372 and CT635 and CT859; (3) CT412 and CT480 and CT869 and CT871; (4) CT050 and CT153 and CT157 and CT165; (5) CT276 and CT296 and CT456 and CT480; (5) CT089 and CT381 and CT396 and CT548; (6) CT635 and CT700 and CT711 and CT859; (7) CT812 and CT869 and CT552 and CT671; (8) CT713 and CT017 and CT043 and CT082; (9) CT266 and CT443 and CT559 and CT597; and (10) CT045 and CT089 and CT396 and CT398 and CT391; or other combinations thereof.

Preferably, the composition of the present invention comprises a combination of *Chlamydia trachomatis* antigens, said combination selected from the group consisting of:
(1) CT016 and CT128 and CT671 and CT262; (2) CT296 and CT372 and CT635 and CT859; (3) CT412 and CT480 and CT869 and CT871; (4) CT050 and CT153 and CT157 and CT165; (5) CT276 and CT296 and CT456 and CT480; (5) CT089 and CT381 and CT396 and CT548; (6) CT635 and CT700 and CT711 and CT859; (7) CT812 and CT869 and CT552 and CT671; (8) CT713 and CT017 and CT043 and CT082; (9) CT266 and CT443 and CT559 and CT597; and and (10) CT045 and CT089 and CT396 and CT398 and CT391; or other combinations thereof; in combination with an immunoregulatory agent which is selected from the group consisting of CFA, Alum, CpG, AlOH, Alum and CpG, AlOH and CpG, and LTK63.

Preferably, the composition of the present invention comprises a combination of *Chlamydia trachomatis* antigens, said combination selected from the group consisting of:
(1) CT016 and CT128 and CT671 and CT262; (2) CT296 and CT372 and CT635 and CT859; (3) CT412 and CT480 and CT869 and CT871; (4) CT050 and CT153 and CT157 and CT165; (5)

CT276 and CT296 and CT456 and CT480; (5) CT089 and CT381 and CT396 and CT548; (6) CT635 and CT700 and CT711 and CT859; (7) CT812 and CT869 and CT552 and CT671; (8) CT713 and CT017 and CT043 and CT082; (9) CT266 and CT443 and CT559 and CT597; and and (10) CT045 and CT089 and CT396 and CT398 and CT391; or other combinations thereof; in combination with Alum and CpG or AlOH and CpG.

Preferably, the composition of the present invention comprises a combination of *Chlamydia trachomatis* antigens, said combination selected from the group consisting of (1) CT242 and CT316; (2)CT467 and CT444; and (3) CT812 and CT082; or other combinations thereof.

Preferably, the composition of the present invention comprises a combination of *Chlamydia* trachomatis antigens, said combination selected from the group consisting of (1) CT242 and CT316; (2)CT467 and CT444; and (3) CT812 and CT082; or other combinations thereof in combination with an immunoregulatory agent which is selected from the group consisting of CFA, Alum, CpG, AlOH, Alum and CpG, AlOH and CpG and LTK63.

Preferably, the composition of the present invention comprises a combination of *Chlamydia trachomatis* antigens, said combination selected from the group consisting of (1) CT242 and CT316; (2)CT467 and CT444; and (3) CT812 and CT082; or other combinations thereof in combination with Alum and CpG or AlOH and CpG.

Preferably the immunogenic compositions of the present invention comprise one or more antigens selected from a "fourth antigen" group consisting of:
(1) CT559 (YscJ); (2) CT600 (Pal); (3) CT541 (Mip); (4) CT623 (CHLPN 76kDA homologue) (5) CT700 (Hypothetical protein). (6) CT266 (Hypothetical protein); (7) CT077 (Hypothetical protein); (8) CT456 (Hypothetical protein); (9) CT165 (Hypothetical protein) and (10) CT713 (PorB).

Preferably the immunogenic compositions of the present invention comprise one or more antigens selected from a "fifth antigen" group consisting of:
(1) CT082 (hypothetical); (2) CT181 (Hypothetical); (3) CT050 (Hypothetical); (4) CT157 (Phospholipase D superfamily); and (5) CT128 (AdK adenylate cyclase).

Preferably the immunogenic compositions of the present invention comprise one or more antigens selected from a "sixth antigen" group consisting of:
: (1) CT153 (Hypothetical); (2) CT262 (Hypothetical); (3) CT276 (Hypothetical); (4) CT296 (Hypothetical); (5) CT372 (Hypothetical); (6) CT412 (PmpA); (7) CT480 (OligoPeptide Binding Protein); (8) CT548 (Hypothetical); (9) CT043 (Hypothetical); (10) CT635 (Hypothetical); (11) CT859 (Metalloprotease);(12) CT671 (Hypothetical); (13) CT016 (Hypothetical); (14) CT017 (Hypothetical); (15) CT043 (Hypothetical); (16) CT082 (Hypothetical); (17)CT548 (Hypothetical); (19) CT089 (Low Calcium Response Element); (20) CT812 (PmpD) and (21) CT869 (PmpE).

FACS analyses, Western Blot analyses and In-vitro neutralisation analyses- carried out as described in the Examples and in WO 03/049762 - demonstrate that proteins in the fourth antigen group, the fifth antigen group and the sixth antigen group are surface-exposed and immunoaccessible proteins and are useful immunogens. These properties are not evident from the sequence alone. In addition, proteins described in the fourth, fifith and sixth antigen groups (as well as the first, second, third and fourth antigen groups) which are described as

"hypothetical" have no known cellular location or biological function and generally, do not have any bactieral homologue, such as *Chlamydia pneumoniae* homologues.

Fusion proteins

The *Chlamydia trachomatis* antigens used in the invention may be present in the composition as individual separate polypeptides. Generally, the recombinant fusion proteins of the present invention are prepared as a GST-fusion protein and/or a His-tagged fusion protein.

However, preferably, at least two (*i.e.* 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20) of the antigens are expressed as a single polypeptide chain (a 'hybrid' polypeptide). Hybrid polypeptides offer two principal advantages: first, a polypeptide that may be unstable or poorly expressed on its own can be assisted by adding a suitable hybrid partner that overcomes the problem; second, commercial manufacture is simplified as only one expression and purification need be employed in order to produce two polypeptides which are both antigenically useful.

The hybrid polypeptide may comprise two or more polypeptide sequences from the first antigen group. Accordingly, the invention includes a composition comprising a first amino acid sequence and a second amino acid sequence, wherein said first and second amino acid sequences are selected from a *Chlamydia trachomatis* antigen or a fragment thereof of the first antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise different epitopes.

The hybrid polypeptide may comprise two or more polypeptide sequences from the second antigen group. Accordingly, the invention includes a composition comprising a first amino acid sequence and a second amino acid sequence, wherein said first and second amino acid sequences are selected from a *Chlamydia trachomatis* antigen or a fragment thereof of the second antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise difference epitopes.

The hybrid polypeptide may comprise one or more polypeptide sequences from the first antigen group and one or more polypeptide sequences from the second antigen group. Accordingly, the invention includes a composition comprising a first amino acid sequence and a second amino acid sequence, said first amino acid sequence selected from a *Chlamydia trachomatis* antigen or a fragment thereof from the first antigen group and said second amino acid sequence selected from a *Chlamydia trachomatis* antigen or a fragment thereof from the second antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise difference epitopes.

The hybrid polypeptide may comprise one or more polypeptide sequences from the first antigen group and one or more polypeptide sequences from the third antigen group. Accordingly, the invention includes a composition comprising a first amino acid sequence and a second amino acid sequence, said first amino acid sequence selected from a *Chlamydia trachomatis* antigen or a fragment thereof from the first antigen group and said second amino acid sequence selected from a *Chlamydia trachomatis* antigen or a fragment thereof from the third antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise difference epitopes.

The hybrid polypeptide may comprise one or more polypeptide sequences from the second antigen group and one or more polypeptide sequences from the third antigen group. Accordingly, the invention includes a composition comprising a first amino acid sequence and a second amino acid sequence, said first amino acid sequence selected from a *Chlamydia trachomatis* antigen or a fragment thereof from the second antigen group and said second amino acid sequence selected from a *Chlamydia trachomatis* antigen or a fragment thereof from the third antigen group. Preferably, the first and second amino acid sequences in the hybrid polypeptide comprise difference epitopes.

Hybrids consisting of amino acid sequences from two, three, four, five, six, seven, eight, nine, or ten *Chlamydia trachomatis* antigens are preferred. In particular, hybrids consisting of amino acid sequences from two, three, four, or five *Chlamydia trachomatis* antigens are preferred. Different hybrid polypeptides may be mixed together in a single formulation. Within such combinations, a *Chlamydia trachomatis* antigen may be present in more than one hybrid polypeptide and/or as a non-hybrid polypeptide. It is preferred, however, that an antigen is present either as a hybrid or as a non-hybrid, but not as both.

Two-antigen hybrids for use in the invention may comprise: (1) PepA & LcrE; (2) PepA & OmpH-like; (3) PepA & L7/L12; (4) PepA & ArtJ; (5) PepA & DnaK; (6) PepA & CT398; (7) PepA & OmcA; (8) PepA & AtoS; (9) PepA & CT547; (10) PepA & Eno; (11) PepA & HrtA; (12) PepA & MurG; (13) LcrE & OmpH-like; (14) LcrE & L7/L12; (15) LcrE & ArtJ; (16) LcrE & DnaK; (17) LcrE & CT398; (18) LcrE & OmcA; (19) LcrE & AtoS; (20) LcrE & CT547; (21) LcrE & Eno; (22) LcrE & HrtA; (23) LcrE & MurG; (24) OmpH-like & L7/L12; (25) OmpH-like & ArtJ; (26) OmpH-like & DnaK; (27) OmpH-like & CT398; (28) OmpH-like & OmcA; (29) OmpH-like & AtoS; (30) OmpH-like & CT547; (31) OmpH-like & Eno; (32) OmpH-like & HrtA; (33) OmpH-like & MurG; (34) L7/L12 & ArtJ; (35) L7/L12 & DnaK; (36) L7/L12 & CT398; (37) L7/L12 & OmcA; (38) L7/L12 & AtoS; (39) L7/L12 & CT547; (40) L7/L12 & Eno; (41) L7/L12 & HrtA; (42) L7/L12 & MurG; (43) ArtJ & DnaK; (44) ArtJ & CT398; (45) ArtJ & OmcA; (46) ArtJ & AtoS; (47) ArtJ & CT547; (48) ArtJ & Eno; (49) ArtJ & HrtA; (50) ArtJ & MurG; (51) DnaK & CT398; (52) DnaK & OmcA; (53) DnaK & AtoS; (54) DnaK & CT547; (55) DnaK & Eno; (56) DnaK & HrtA; (57) DnaK & MurG; (58) CT398 & OmcA; (59) CT398 & AtoS; (60) CT398 & CT547; (61) CT398 & Eno; (62) CT398 & HrtA; (63) CT398 & MurG; (64) OmcA & AtoS; (65) OmcA & CT547; (66) OmcA & Eno; (67) OmcA & HrtA; (68) OmcA & MurG; (69) AtoS & CT547; (70) AtoS & Eno; (71) AtoS & HrtA; (72) AtoS & MurG; (73) CT547 & Eno; (74) CT547 & HrtA; (75) CT547 & MurG; (76) Eno & HrtA; (77) Eno & MurG; (78) HrtA & MurG or (79) PmpD (CT812) and Hypothetical (CT082).

Two antigen hybrids for use in the present invention may also comprise combinations of antigens selected from the third, fourth, fifth and sixth antigen groups.

Hybrid polypeptides can be represented by the formula NH_2-A-{-X-L-}$_n$-B-COOH, wherein: X is an amino acid sequence of a *Chlamydia trachomatis* antigen or a fragment thereof from the first antigen group, the second antigen group or the third antigen group; L is an optional linker amino acid sequence; A is an optional N-terminal amino acid sequence; B is an optional C-terminal amino acid sequence; and *n* is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15.

If a -X- moiety has a leader peptide sequence in its wild-type form, this may be included or omitted in the hybrid protein. In some embodiments, the leader peptides will be deleted except for that of the -X- moiety located at the N-terminus of the hybrid protein *i.e.* the leader peptide of

X_1 will be retained, but the leader peptides of X_2 ... X_n will be omitted. This is equivalent to deleting all leader peptides and using the leader peptide of X_1 as moiety -A-.

For each *n* instances of {-X-L-}, linker amino acid sequence -L- may be present or absent. For instance, when *n*=2 the hybrid may be NH_2-X_1-L_1-X_2-L_2-COOH, NH_2-X_1-X_2-COOH, NH_2-X_1-L_1-X_2-COOH, NH_2-X_1-X_2-L_2-COOH, *etc.* Linker amino acid sequence(s) -L- will typically be short (*e.g.* 20 or fewer amino acids *i.e.* 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples comprise short peptide sequences which facilitate cloning, poly-glycine linkers (*i.e.* comprising Gly_n where n = 2, 3, 4, 5, 6, 7, 8, 9, 10 or more), and histidine tags (*i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable linker amino acid sequences will be apparent to those skilled in the art. A useful linker is GSGGGG (SEQ ID 1), with the Gly-Ser dipeptide being formed from a *Bam*HI restriction site, thus aiding cloning and manipulation, and the $(Gly)_4$ tetrapeptide being a typical poly-glycine linker.

-A- is an optional N-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include leader sequences to direct protein trafficking, or short peptide sequences which facilitate cloning or purification (*e.g.* histidine tags *i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable N-terminal amino acid sequences will be apparent to those skilled in the art. If X_1 lacks its own N-terminus methionine, -A- is preferably an oligopeptide (*e.g.* with 1, 2, 3, 4, 5, 6, 7 or 8 amino acids) which provides a N-terminus methionine.

-B- is an optional C-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include sequences to direct protein trafficking, short peptide sequences which facilitate cloning or purification (*e.g.* comprising histidine tags *i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more), or sequences which enhance protein stability. Other suitable C-terminal amino acid sequences will be apparent to those skilled in the art. Most preferably, *n* is 2 or 3.

The invention also provides nucleic acid encoding hybrid polypeptides of the invention. Furthermore, the invention provides nucleic acid which can hybridise to this nucleic acid, preferably under "high stringency" conditions (*e.g.* 65°C in a 0.1xSSC, 0.5% SDS solution).

Polypeptides of the invention can be prepared by various means (*e.g.* recombinant expression, purification from cell culture, chemical synthesis, *etc.*) and in various forms (*e.g.* native, fusions, non-glycosylated, lipidated, *etc.*). They are preferably prepared in substantially pure form (*i.e.* substantially free from other chlamydial or host cell proteins).

Nucleic acid according to the invention can be prepared in many ways (*e.g.* by chemical synthesis, from genomic or cDNA libraries, from the organism itself, *etc.*) and can take various forms (*e.g.* single stranded, double stranded, vectors, probes, *etc.*). They are preferably prepared in substantially pure form (*i.e.* substantially free from other chlamydial or host cell nucleic acids).

The term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones (*e.g.* phosphorothioates, *etc.*), and also peptide nucleic acids (PNA), *etc.* The invention includes nucleic acid comprising sequences complementary to those described above (*e.g.* for antisense or probing purposes).

The invention also provides a process for producing a polypeptide of the invention, comprising the step of culturing a host cell transformed with nucleic acid of the invention under conditions which induce polypeptide expression.

The invention provides a process for producing a polypeptide of the invention, comprising the step of synthesising at least part of the polypeptide by chemical means.

The invention provides a process for producing nucleic acid of the invention, comprising the step of amplifying nucleic acid using a primer-based amplification method (*e.g.* PCR).

The invention provides a process for producing nucleic acid of the invention, comprising the step of synthesising at least part of the nucleic acid by chemical means.

Strains

Preferred polypeptides of the invention comprise an amino acid sequence found in *C.trachomatis* serovar D, or in one or more of an epidemiologically prevalent serotype.
Where hybrid polypeptides are used, the individual antigens within the hybrid (*i.e.* individual -X- moieties) may be from one or more strains. Where n=2, for instance, X_2 may be from the same strain as X_1 or from a different strain. Where n=3, the strains might be (i) $X_1=X_2=X_3$ (ii) $X_1=X_2\neq X_3$ (iii) $X_1\neq X_2=X_3$ (iv) $X_1\neq X_2\neq X_3$ or (v) $X_1=X_3\neq X_2$, *etc.*

Heterologous host

Whilst expression of the polypeptides of the invention may take place in *Chlamydia*, the invention preferably utilises a heterologous host. The heterologous host may be prokaryotic (*e.g.* a bacterium) or eukaryotic. It is preferably *E.coli*, but other suitable hosts include *Bacillus subtilis, Vibrio cholerae, Salmonella typhi, Salmonella typhimurium, Neisseria lactamica, Neisseria cinerea, Mycobacteria* (*e.g. M.tuberculosis*), yeasts, *etc.*

Immunogenic compositions and medicaments

Compositions of the invention are preferably immunogenic compositions, and are more preferably vaccine compositions. The pH of the composition is preferably between 6 and 8, preferably about 7. The pH may be maintained by the use of a buffer. The composition may be sterile and/or pyrogen-free. The composition may be isotonic with respect to humans.
Vaccines according to the invention may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat infection), but will typically be prophylactic. Accordingly, the invention includes a method for the therapeutic or prophylactic treatment of *Chlamydia trachomatis* infection in an animal susceptible to chlamydial infection comprising administering to said animal a therapeutic or prophylactic amount of the immunogenic compositions of the invention.
Preferably, the immunogenic composition comprises a combination of *Chlamydia trachomatis* antigens, said combination selected from the group consisting of two, three, four, or all five *Chlamydia trachomatis* antigens of the first antigen group. Still more preferably, the combination consists of all five *Chlamydia trachomatis* antigens of the first antigen group.

Alternatively, the immunogenic composition comprises a combination of *Chlamydia trachomatis* antigens, said combination selected from the group consisting of two, three, four, five, six, seven,

eight, nine, ten, eleven, twelve, or thirteen *Chlamydia trachomatis* antigens selected from the second antigen group. Preferably, the combination is selected from the group consisting of three, four, or five *Chlamydia trachomatis* antigens selected from the second antigen group. Still more preferably, the combination consists of five *Chlamydia trachomatis* antigens selected from the second antigen group.

Alternatively, the immunogenic composition comprises a combination of *Chlamydia trachomatis* antigens, said combination consisting of two, three, four, or five *Chlamydia trachomatis* antigens of the first antigen group and one, two, three, four, five or six *Chlamydia trachomatis* antigens of the third antigen group. Preferably, the combination consists of three, four or five *Chlamydia trachomatis* antigens of the first antigen group and one, two, three, four, five or six *Chlamydia trachomatis* antigens of the third antigen group.

Alternatively, the immunigenic composition comprises a combination of *Chlamydia trachomatis* antigens, said combination consisting of two, three, four ,five, six, seven, eight, nine, ten, eleven, twelve or thirteen *Chlamydia trachomatis* antigens of the second antigen group and one, two, three, four, five or six *Chlamydia trachomatis* antigens of the third antigen group. Preferably, the combination is selected from the group consisting of three, four, or five *Chlamydia trachomatis* antigens from the second antigen group and three, four or five *Chlamydia trachomatis* from the third antigen group. Still more preferably, the combination consists of five *Chlamydia trachomatis* antigens from the second antigen group and three, four or five *Chlamydia trachomatis* antigens of the third antigen group.

Alternatively, the immunigenic composition comprises a combination of *Chlamydia trachomatis* antigens, said combination consisting of two, three, four ,five, six, seven, eight, nine or ten *Chlamydia trachomatis* antigens of the fourth antigen group and one, two, three, four or five *Chlamydia trachomatis* antigens of the fifth antigen group and one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty or twentyone antigens of the sixth antigen group.. Preferably, the combination is selected from the group consisting of three, four, or five *Chlamydia trachomatis* antigens from the fourth antigen group and three, four or five *Chlamydia trachomatis* from the fifth antigen group. Still more preferably, the combination consists of five *Chlamydia trachomatis* antigens from the fourth antigen group and three, four or five *Chlamydia trachomatis* antigens of the fifth antigen group.

The invention also provides a composition of the invention for use as a medicament. The medicament is preferably able to raise an immune response in a mammal (*i.e.* it is an immunogenic composition) and is more preferably a vaccine.

The invention also provides the use of the compositions of the invention in the manufacture of a medicament for raising an immune response in a mammal. The medicament is preferably a vaccine.

The invention also provides for a kit comprising a first component comprising a combination of *Chlamydia trachomatis* antigens. The combination of *Chlamydia trachomatis* antigens may be one or more of the immunogenic compositions of the invention. The kit may further include a second component comprising one or more of the following: instructions, syringe or other delivery device, adjuvant, or pharmaceutically acceptable formulating solution.

The invention also provides a delivery device pre-filled with the immunogenic compositions of the invention.

The invention also provides a method for raising an immune response in a mammal comprising the step of administering an effective amount of a composition of the invention. The immune response is preferably protective and preferably involves antibodies and/or cell-mediated immunity. The method may raise a booster response.

The mammal is preferably a human. Where the vaccine is for prophylactic use, the human is preferably a child (*e.g.* a toddler or infant) or a teenager; where the vaccine is for therapeutic use, the human is preferably a teenager or an adult. A vaccine intended for children may also be administered to adults *e.g.* to assess safety, dosage, immunogenicity, *etc.*

These uses and methods are preferably for the prevention and/or treatment of a disease caused by a *Chlamydia* (*e.g.* trachoma, pelvic inflammatory disease, epididymitis, infant pneumonia, *etc.*). The compositions may also be effective against *C.pneumoniae*.

One way of checking efficacy of therapeutic treatment involves monitoring *C.trachomatis* infection after administration of the composition of the invention. One way of checking efficacy of prophylactic treatment involves monitoring immune responses against the *Chlamydia trachomatis* antigens in the compositions of the invention after administration of the composition.

The vaccine compositions of the present invention can be evaluated in *in vitro* and *in vivo* animal models prior to host, e.g., human, administration. For example, *in vitro* neutralization by Peterson et al (1988) is suitable for testing vaccine compositions directed toward *Chlamydia trachomatis*.

One example of such an *in vitro* test is described as follows. Hyper-immune antisera is diluted in PBS containing 5% guinea pig serum, as a complement source. *Chlamydia trachomatis* (10^4 IFU; inclusion forming units) are added to the antisera dilutions. The antigen-antibody mixtures are incubated at 37°C for 45 minutes and inoculated into duplicate confluent Hep-2 or HeLa cell monolayers contained in glass vials (e.g., 15 by 45 mm), which have been washed twice with PBS prior to inoculation. The monolayer cells are infected by centrifugation at 1000X g for 1 hour followed by stationary incubation at 37°C for 1 hour. Infected monolayers are incubated for 48 or 72 hours , fixed and stained with Chlamydia specific antibody, such as anti-MOMP. Inclusion-bearing cells are counted in ten fields at a magnification of 200X. Neutralization titer is assigned on the dilution that gives 50% inhibition as compared to control monolayers/IFU.

The efficacy of vaccine compositions can also be determined *in vivo* by challenging animal models of *Chlamydia trachomatis* infection, *e.g.*, guinea pigs or mice, with the vaccine compositions. For example, *in vivo* vaccine composition challenge studies in the guinea pig model of *Chlamydia trachomatis* infection can be performed. A description of one example of this type of approach follows. Female guinea pigs weighing 450 – 500 g are housed in an environmentally controlled room with a 12 hour light-dark cycle and immunized with vaccine compositions via a variety of immunization routes. Post-vaccination, guinea pigs are infected in the genital tract with the agent of guinea pig inclusion conjunctivitis (GPIC), which has been grown in HeLa or McCoy cells (Rank et al. (1988)). Each animal receives approximately $1.4x10^7$ inclusion forming units (IFU) contained in 0.05 ml of sucrose-phosphate-glutamate buffer, pH 7.4 (Schacter, 1980). The course of infection monitored by determining the

percentage of inclusion-bearing cells by indirect immunofluorescence with GPIC specific antisera, or by Giemsa-stained smear from a scraping from the genital tract (Rank et al 1988). Antibody titers in the serum is determined by an enzyme-linked immunosorbent assay.

Alternatively, *in vivo* vaccine compositions challenge studies can be performed in the murine model of *Chlamydia trachomatis* (Morrison et al 1995). A description of one example of this type of approach is as follows. Female mice 7 to 12 weeks of age receive 2.5 mg of depoprovera subcutaneously at 10 and 3 days before vaginal infection. Post-vaccination, mice are infected in the genital tract with 1,500 inclusion-forming units of *Chlamydia trachomatis* contained in 5ml of sucrose-phosphate-glutamate buffer, pH 7.4. The course of infection is monitored by determining the percentage of inclusion-bearing cells by indirect immunofluorescence with *Chlamydia trachomatis* specific antisera, or by a Giemsa-stained smear from a scraping from the genital tract of an infected mouse. The presence of antibody titers in the serum of a mouse is determined by an enzyme-linked immunosorbent assay.

Compositions of the invention will generally be administered directly to a patient. Direct delivery may be accomplished by parenteral injection (*e.g.* subcutaneously, intraperitoneally, intravenously, intramuscularly, or to the interstitial space of a tissue), or by rectal, oral (*e.g.* tablet, spray), vaginal, topical, transdermal {*e.g.* see ref. l} or transcutaneous {*e.g.* see refs. li & lii}, intranasal {*e.g.* see ref. liii}, ocular, aural, pulmonary or other mucosal administration. The invention may be used to elicit systemic and/or mucosal immunity.

Dosage treatment can be a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunisation schedule and/or in a booster immunisation schedule. In a multiple dose schedule the various doses may be given by the same or different routes *e.g.* a parenteral prime and mucosal boost, a mucosal prime and parenteral boost, *etc.*

Chlamydial infections affect various areas of the body and so the compositions of the invention may be prepared in various forms. For example, the compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared (*e.g.* a lyophilised composition). The composition may be prepared for topical administration *e.g.* as an ointment, cream or powder. The composition may be prepared for oral administration *e.g.* as a tablet or capsule, as a spray, or as a syrup (optionally flavoured). The composition may be prepared for pulmonary administration *e.g.* as an inhaler, using a fine powder or a spray. The composition may be prepared as a suppository or pessary. The composition may be prepared for nasal, aural or ocular administration *e.g.* as drops. The composition may be in kit form, designed such that a combined composition is reconstituted just prior to administration to a patient. Such kits may comprise one or more antigens in liquid form and one or more lyophilised antigens.

Immunogenic compositions used as vaccines comprise an immunologically effective amount of antigen(s), as well as any other components, as needed. By 'immunologically effective amount', it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (*e.g.* non-human primate, primate, *etc.*), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is

expected that the amount will fall in a relatively broad range that can be determined through routine trials.

Further components of the composition

The composition of the invention will typically, in addition to the components mentioned above, comprise one or more 'pharmaceutically acceptable carriers', which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. The vaccines may also contain diluents, such as water, saline, glycerol, *etc.* Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present. A thorough discussion of pharmaceutically acceptable excipients is available in reference liv.

ImmunoRegulatory Agents

Vaccines of the present invention may be administered in conjunction with other immunoregulatory agents. In particular, compositions will usually include an adjuvant. Adjuvants for use with the invention include, but are not limited to, one or more of the following set forth below:

A. Mineral Containing Compositions

Mineral containing compositions suitable for use as adjuvants in the invention include mineral salts, such as aluminum salts and calcium salts. The invention includes mineral salts such as hydroxides (*e.g.* oxyhydroxides), phosphates (*e.g.* hydroxyphosphates, orthophosphates), sulfates, *etc.* (*e.g.* see chapters 8 & 9 of *Vaccine Design...* (1995) eds. Powell & Newman. ISBN: 030644867X. Plenum.), or mixtures of different mineral compounds (*e.g.* a mixture of a phosphate and a hydroxide adjuvant, optionally with an excess of the phosphate), with the compounds taking any suitable form (*e.g.* gel, crystalline, amorphous, *etc.*), and with adsorption to the salt(s) being preferred. The mineral containing compositions may also be formulated as a particle of metal salt (WO00/23105).

Aluminum salts may be included in vaccines of the invention such that the dose of Al^{3+} is between 0.2 and 1.0 mg per dose.

Preferably the adjuvant is alum, preferably AlOH.

B. Oil-Emulsions

Oil-emulsion compositions suitable for use as adjuvants in the invention include squalene-water emulsions, such as MF59 (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer). See WO90/14837. See also, Frey et al., "Comparison of the safety, tolerability, and immunogenicity of a MF59-adjuvanted influenza vaccine and a non-adjuvanted influenza vaccine in non-elderly adults", Vaccine (2003) 21:4234-4237. MF59 is used as the adjuvant in the FLUAD™ influenza virus trivalent subunit vaccine.

Particularly preferred adjuvants for use in the compositions are submicron oil-inwater emulsions. Preferred submicron oil-in-water emulsions for use herein are squalene/water emulsions optionally containing varying amounts of MTP-PE, such as a submicron oil-in-water emulsion containing 4-5% w/v squalene, 0.25-1.0% w/v Tween 80 ™ (polyoxyelthylenesorbitan monooleate), and/or 0.25-1.0% Span 85™ (sorbitan trioleate), and, optionally, N-acetylmuramyl-

L-alanyl-D-isogluatminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-huydroxyphosphophoryloxy)-ethylamine (MTP-PE), for example, the submicron oil-in-water emulsion known as "MF59" (International Publication No. WO90/14837; US Patent Nos. 6,299,884 and 6,451,325, incorporated herein by reference in their entireties; and Ott et al., "MF59 -- Design and Evaluation of a Safe and Potent Adjuvant for Human Vaccines" in *Vaccine Design: The Subunit and Adjuvant Approach* (Powell, M.F. and Newman, M.J. eds.) Plenum Press, New York, 1995, pp. 277-296). MF59 contains 4-5% w/v Squalene (e.g. 4.3%), 0.25-0.5% w/v Tween 80™, and 0.5% w/v Span 85™ and optionally contains various amounts of MTP-PE, formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA). For example, MTP-PE may be present in an amount of about 0-500 μg/dose, more preferably 0-250 μg/dose and most preferably, 0-100 μg/dose. As used herein, the term "MF59-0" refers to the above submicron oil-in-water emulsion lacking MTP-PE, while the term MF59-MTP denotes a formulation that contains MTP-PE. For instance, "MF59-100" contains 100 μg MTP-PE per dose, and so on. MF69, another submicron oil-in-water emulsion for use herein, contains 4.3% w/v squalene, 0.25% w/v Tween 80™, and 0.75% w/v Span 85™ and optionally MTP-PE. Yet another submicron oil-in-water emulsion is MF75, also known as SAF, containing 10% squalene, 0.4% Tween 80™, 5% pluronic-blocked polymer L121, and thr-MDP, also microfluidized into a submicron emulsion. MF75-MTP denotes an MF75 formulation that includes MTP, such as from 100-400 μg MTP-PE per dose.

Submicron oil-in-water emulsions, methods of making the same and immunostimulating agents, such as muramyl peptides, for use in the compositions, are described in detail in International Publication No. WO90/14837 and US Patent Nos. 6,299,884 and 6,45 1,325, incorporated herein by reference in their entireties. Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) may also be used as adjuvants in the invention.

C. Saponin Formulations

Saponin formulations, may also be used as adjuvants in the invention. Saponins are a heterologous group of sterol glycosides and triterpenoid glycosides that are found in the bark, leaves, stems, roots and even flowers of a wide range of plant species. Saponin from the bark of the *Quillaia saponaria* Molina tree have been widely studied as adjuvants. Saponin can also be commercially obtained from *Smilax ornata* (sarsaprilla), *Gypsophilla paniculata* (brides veil), and *Saponaria officianalis* (soap root). Saponin adjuvant formulations include purified formulations, such as QS21, as well as lipid formulations, such as ISCOMs.

Saponin compositions have been purified using High Performance Thin Layer Chromatography (HP-LC) and Reversed Phase High Performance Liquid Chromatography (RP-HPLC). Specific purified fractions using these techniques have been identified, including QS7, QS17, QS18, QS21, QH-A, QH-B and QH-C. Preferably, the saponin is QS21. A method of production of QS21 is disclosed in US Patent No. 5,057,540. Saponin formulations may also comprise a sterol, such as cholesterol (see WO96/33739).

Combinations of saponins and cholesterols can be used to form unique particles called Immunostimulating Complexs (ISCOMs). ISCOMs typically also include a phospholipid such as phosphatidylethanolamine or phosphatidylcholine. Any known saponin can be used in ISCOMs. Preferably, the ISCOM includes one or more of Quil A, QHA and QHC. ISCOMs are further described in EP0109942, WO96/11711 and WO96/33739. Optionally, the ISCOMS may be devoid of additional detergent. See WO00/07621.

A review of the development of saponin based adjuvants can be found at Barr, et al., "ISCOMs and other saponin based adjuvants", Advanced Drug Delivery Reviews (1998) 32:247-271. See also Sjolander, et al., "Uptake and adjuvant activity of orally delivered saponin and ISCOM vaccines", Advanced Drug Delivery Reviews (1998) 32:321-338.

D. Virosomes and Virus Like Particles (VLPs)

Virosomes and Virus Like Particles (VLPs) can also be used as adjuvants in the invention. These structures generally contain one or more proteins from a virus optionally combined or formulated with a phospholipid. They are generally non-pathogenic, non-replicating and generally do not contain any of the native viral genome. The viral proteins may be recombinantly produced or isolated from whole viruses. These viral proteins suitable for use in virosomes or VLPs include proteins derived from influenza virus (such as HA or NA), Hepatitis B virus (such as core or capsid proteins), Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-Mouth Disease virus, Retrovirus, Norwalk virus, human Papilloma virus, HIV, RNA-phages, Qß-phage (such as coat proteins), GA-phage, fr-phage, AP205 phage, and Ty (such as retrotransposon Ty protein p1). VLPs are discussed further in WO03/024480, WO03/024481, and Niikura et al., "Chimeric Recombinant Hepatitis E Virus-Like Particles as an Oral Vaccine Vehicle Presenting Foreign Epitopes", Virology (2002) 293:273-280; Lenz et al., "Papillomarivurs-Like Particles Induce Acute Activation of Dendritic Cells", Journal of Immunology (2001) 5246-5355; Pinto, et al., "Cellular Immune Responses to Human Papillomavirus (HPV)-16 L1 Healthy Volunteers Immunized with Recombinant HPV-16 L1 Virus-Like Particles", Journal of Infectious Diseases (2003) 188:327-338; and Gerber et al., "Human Papillomavrisu Virus-Like Particles Are Efficient Oral Immunogens when Coadministered with Escherichia coli Heat-Labile Entertoxin Mutant R192G or CpG", Journal of Virology (2001) 75(10):4752-4760. Virosomes are discussed further in, for example, Gluck et al., "New Technology Platforms in the Development of Vaccines for the Future", Vaccine (2002) 20:B10 –B16. Immunopotentiating reconstituted influenza virosomes (IRIV) are used as the subunit antigen delivery system in the intranasal trivalent INFLEXAL™ product {Mischler & Metcalfe (2002) *Vaccine* 20 Suppl 5:B17-23} and the INFLUVAC PLUS™ product.

E. Bacterial or Microbial Derivatives

Adjuvants suitable for use in the invention include bacterial or microbial derivatives such as:

(1) *Non-toxic derivatives of enterobacterial lipopolysaccharide (LPS)*
Such derivatives include Monophosphoryl lipid A (MPL) and 3-O-deacylated MPL (3dMPL). 3dMPL is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. A preferred "small particle" form of 3 De-O-acylated monophosphoryl lipid A is disclosed in EP 0 689 454. Such "small particles" of 3dMPL are small enough to be sterile filtered through a 0.22 micron membrane (see EP 0 689 454). Other non-toxic LPS derivatives include monophosphoryl lipid A mimics, such as aminoalkyl glucosaminide phosphate derivatives *e.g.* RC-529. See Johnson *et al.* (1999) *Bioorg Med Chem Lett* 9:2273-2278.

(2) *Lipid A Derivatives*
Lipid A derivatives include derivatives of lipid A from *Escherichia coli* such as OM-174. OM-174 is described for example in Meraldi et al., "OM-174, a New Adjuvant with a Potential for Human Use, Induces a Protective Response with Administered with the Synthetic C-Terminal Fragment 242-310 from the circumsporozoite protein of Plasmodium berghei", Vaccine (2003) 21:2485-2491; and Pajak, et al., "The Adjuvant OM-174 induces both the migration and maturation of murine dendritic cells in vivo", Vaccine (2003) 21:836-842.

(3) *Immunostimulatory oligonucleotides*
Immunostimulatory oligonucleotides suitable for use as adjuvants in the invention include nucleotide sequences containing a CpG motif (a sequence containing an unmethylated cytosine followed by guanosine and linked by a phosphate bond). Bacterial double stranded RNA or oligonucleotides containing palindromic or poly(dG) sequences have also been shown to be immunostimulatory.

The CpG's can include nucleotide modifications/analogs such as phosphorothioate modifications and can be double-stranded or single-stranded. Optionally, the guanosine may be replaced with an analog such as 2'-deoxy-7-deazaguanosine. See Kandimalla, et al., "Divergent synthetic nucleotide motif recognition pattern: design and development of potent immunomodulatory oligodeoxyribonucleotide agents with distinct cytokine induction profiles", Nucleic Acids Research (2003) 31(9): 2393-2400; WO02/26757 and WO99/62923 for examples of possible analog substitutions. The adjuvant effect of CpG oligonucleotides is further discussed in Krieg, "CpG motifs: the active ingredient in bacterial extracts?", Nature Medicine (2003) 9(7): 831-835; McCluskie, et al., "Parenteral and mucosal prime-boost immunization strategies in mice with hepatitis B surface antigen and CpG DNA", FEMS Immunology and Medical Microbiology (2002) 32:179-185; WO98/40100; US Patent No. 6,207,646; US Patent No. 6,239,116 and US Patent No. 6,429,199.

The CpG sequence may be directed to TLR9, such as the motif GTCGTT or TTCGTT. See Kandimalla, et al., "Toll-like receptor 9: modulation of recognition and cytokine induction by novel synthetic CpG DNAs", Biochemical Society Transactions (2003) 31 (part 3): 654-658. The CpG sequence may be specific for inducing a Th1 immune response, such as a CpG-A ODN, or it may be more specific for inducing a B cell response, such a CpG-B ODN. CpG-A and CpG-B ODNs are discussed in Blackwell, et al., "CpG-A-Induced Monocyte IFN-gamma-Inducible Protein-10 Production is Regulated by Plasmacytoid Dendritic Cell Derived IFN-alpha", J. Immunol. (2003) 170(8):4061-4068; Krieg, "From A to Z on CpG", TRENDS in Immunology (2002) 23(2): 64-65 and WO01/95935. Preferably, the CpG is a CpG-A ODN.

Preferably, the CpG oligonucleotide is constructed so that the 5' end is accessible for receptor recognition. Optionally, two CpG oligonucleotide sequences may be attached at their 3' ends to form "immunomers". See, for example, Kandimalla, et al., "Secondary structures in CpG oligonucleotides affect immunostimulatory activity", BBRC (2003) 306:948-953; Kandimalla, et al., "Toll-like receptor 9: modulation of recognition and cytokine induction by novel synthetic GpG DNAs", Biochemical Society Transactions (2003) 31(part 3):664-658; Bhagat et al., "CpG penta- and hexadeoxyribonucleotides as potent immunomodulatory agents" BBRC (2003) 300:853-861 and WO03/035836.

Preferably the adjuvant is CpG. Even more preferably, the adjuvant is Alum and CpG or AlOH and CpG.

(4) *ADP-ribosylating toxins and detoxified derivatives thereof.*
Bacterial ADP-ribosylating toxins and detoxified derivatives thereof may be used as adjuvants in the invention. Preferably, the protein is derived from *E. coli* (i.e., E. coli heat labile enterotoxin "LT), cholera ("CT"), or pertussis ("PT"). The use of detoxified ADP-ribosylating toxins as mucosal adjuvants is described in WO95/17211 and as parenteral adjuvants in WO98/42375. Preferably, the adjuvant is a detoxified LT mutant such as LT-K63, LT-R72, and LTR192G. The use of ADP-ribosylating toxins and detoxified derivaties thereof, particularly LT-K63 and LT-R72, as adjuvants can be found in the following references, each of which is specifically incorporated by reference herein in their entirety: Beignon, et al., "The LTR72 Mutant of Heat-

Labile Enterotoxin of Escherichia coli Enahnces the Ability of Peptide Antigens to Elicit CD4+ T Cells and Secrete Gamma Interferon after Coapplication onto Bare Skin", Infection and Immunity (2002) 70(6):3012-3019; Pizza, et al., "Mucosal vaccines: non toxic derivatives of LT and CT as mucosal adjuvants", Vaccine (2001) 19:2534-2541; Pizza, et al., "LTK63 and LTR72, two mucosal adjuvants ready for clinical trials" Int. J. Med. Microbiol (2000) 290(4-5):455-461; Scharton-Kersten et al., "Transcutaneous Immunization with Bacterial ADP-Ribosylating Exotoxins, Subunits and Unrelated Adjuvants", Infection and Immunity (2000) 68(9):5306-5313; Ryan et al., "Mutants of Escherichia coli Heat-Labile Toxin Act as Effective Mucosal Adjuvants for Nasal Delivery of an Acellular Pertussis Vaccine: Differential Effects of the Nontoxic AB Complex and Enzyme Activity on Th1 and Th2 Cells" Infection and Immunity (1999) 67(12):6270-6280; Partidos et al., "Heat-labile enterotoxin of Escherichia coli and its site-directed mutant LTK63 enhance the proliferative and cytotoxic T-cell responses to intranasally co-immunized synthetic peptides", Immunol. Lett. (1999) 67(3):209-216; Peppoloni et al., "Mutants of the Escherichia coli heat-labile enterotoxin as safe and strong adjuvants for intranasal delivery of vaccines", Vaccines (2003) 2(2):285-293; and Pine et al., (2002) "Intranasal immunization with influenza vaccine and a detoxified mutant of heat labile enterotoxin from Escherichia coli (LTK63)" J. Control Release (2002) 85(1-3):263-270. Numerical reference for amino acid substitutions is preferably based on the alignments of the A and B subunits of ADP-ribosylating toxins set forth in Domenighini et al., Mol. Microbiol (1995) 15(6):1165-1167, specifically incorporated herein by reference in its entirety.

Preferably the adjuvant is LTK63. Preferably the adjuvant is LTK72.

F. Bioadhesives and Mucoadhesives

Bioadhesives and mucoadhesives may also be used as adjuvants in the invention. Suitable bioadhesives include esterified hyaluronic acid microspheres (Singh *et al.* (2001) *J. Cont. Rele.* 70:267-276) or mucoadhesives such as cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrollidone, polysaccharides and carboxymethylcellulose. Chitosan and derivatives thereof may also be used as adjuvants in the invention. E.g. WO99/27960.

G. Microparticles

Microparticles may also be used as adjuvants in the invention. Microparticles (*i.e.* a particle of ~100nm to ~150μm in diameter, more preferably ~200nm to ~30μm in diameter, and most preferably ~500nm to ~10μm in diameter) formed from materials that are biodegradable and non-toxic (*e.g.* a poly(α-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, *etc.*), with poly(lactide-co-glycolide) are preferred, optionally treated to have a negatively-charged surface (*e.g.* with SDS) or a positively-charged surface (*e.g.* with a cationic detergent, such as CTAB).

H. Liposomes

Examples of liposome formulations suitable for use as adjuvants are described in US Patent No. *6,090,406, US Patent No. 5,916,588, and EP 0 626 169.*

L Polyoxyethylene ether and Polyoxyethylene Ester Formulations

Adjuvants suitable for use in the invention include polyoxyethylene ethers and polyoxyethylene esters. WO99/52549. Such formulations further include polyoxyethylene sorbitan ester surfactants in combination with an octoxynol (WO01/21207) as well as polyoxyethylene alkyl

ethers or ester surfactants in combination with at least one additional non-ionic surfactant such as an octoxynol (WO01/21152).

Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether (laureth 9), polyoxyethylene-9-steoryl ether, polyoxytheylene-8-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether.

J. Polyphosphazene (PCPP)

PCPP formulations are described, for example, in Andrianov et al., "Preparation of hydrogel microspheres by coacervation of aqueous polyphophazene solutions", Biomaterials (1998) 19(1-3):109-115 and Payne et al., "Protein Release from Polyphosphazene Matrices", Adv. Drug. Delivery Review (1998) 31(3):185-196.

K. Muramyl peptides

Examples of muramyl peptides suitable for use as adjuvants in the invention include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-l-alanyl-d-isoglutamine (nor-MDP), and N-acetylmuramyl-l-alanyl-d-isoglutaminyl-l-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE).

L. Imidazoquinolone Compounds.

Examples of imidazoquinolone compounds suitable for use adjuvants in the invention include Imiquamod and its homologues, described further in Stanley, "Imiquimod and the imidazoquinolones: mechanism of action and therapeutic potential" Clin Exp Dermatol (2002) 27(7):571-577 and Jones, "Resiquimod 3M", Curr Opin Investig Drugs (2003) 4(2):214-218.

The invention may also comprise combinations of aspects of one or more of the adjuvants identified above. For example, the following adjuvant compositions may be used in the invention:

(1) a saponin and an oil-in-water emulsion (WO99/11241);

(2) a saponin (e.g.., QS21) + a non-toxic LPS derivative (e.g. 3dMPL) (see WO94/00153);

(3) a saponin (e.g.., QS21) + a non-toxic LPS derivative (e.g. 3dMPL) + a cholesterol;

(4) a saponin (*e.g.* QS21) + 3dMPL + IL-12 (optionally + a sterol) (WO98/57659);

(5) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (See European patent applications 0835318, 0735898 and 0761231);

(6) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion.

(7) Ribi™ adjuvant system (RAS), (Ribi Immunochem) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); and

(8) one or more mineral salts (such as an aluminum salt) + a non-toxic derivative of LPS (such as 3dPML).

Aluminum salts and MF59 are preferred adjuvants for use with injectable influenza vaccines. Bacterial toxins and bioadhesives are preferred adjuvants for use with mucosally-delivered vaccines, such as nasal vaccines.

M. Human Immunomodulators

Human immunomodulators suitable for use as adjuvants in the invention include cytokines, such as interleukins (*e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (*e.g.* interferon-γ), macrophage colony stimulating factor, and tumor necrosis factor.

Further antigens

The compositions of the invention may further comprise antigen derived from one or more sexually transmitted diseases in addition to *Chlamydia trachomatis*. Preferably the antigen is derived from one or more of the following sexually transmitted diseases: *N.gonorrhoeae* {*e.g.* lv, lvi, lvii, lviii}; human papiloma virus; *Treponema pallidum;* herpes simplex virus (HSV-1 or HSV-2); HIV (HIV-1 or HIV-2); and *Haemophilus ducreyi.*

A preferred composition comprises: (1) at least *t* of the *Chlamydia trachomatis* antigens from either the first antigen group or the second antigen group, where *t* is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13, preferably *t* is five; (2) one or more antigens from another sexually transmitted disease. Preferably, the sexually transmitted disease is selected from the group consisting of herpes simplex virus, preferably HSV-1 and/or HSV-2; human papillomavirus; *N.gonorrhoeae*; *Treponema pallidum*; and *Haemophilus ducreyi.* These compositions can thus provide protection against the following sexually-transmitted diseases: chlamydia, genital herpes, genital warts, gonorrhoea, syphilis and chancroid (See, Ref. lix).

Where a saccharide or carbohydrate antigen is used, it is preferably conjugated to a carrier protein in order to enhance immunogenicity {*e.g.* refs. lx to lxix}. Preferred carrier proteins are bacterial toxins or toxoids, such as diphtheria or tetanus toxoids. The CRM_{197} diphtheria toxoid is particularly preferred {lxx}. Other carrier polypeptides include the *N.meningitidis* outer membrane protein {lxxi}, synthetic peptides {lxxii, lxxiii}, heat shock proteins {lxxiv, lxxv}, pertussis proteins {lxxvi, lxxvii}, protein D from *H.influenzae* {lxxviii}, cytokines {lxxix}, lymphokines, hormones, growth factors, toxin A or B from *C.difficile* {lxxx}, iron-uptake proteins {lxxxi}, *etc.* Where a mixture comprises capsular saccharides from both serogroups A and C, it may be preferred that the ratio (w/w) of MenA saccharide:MenC saccharide is greater than 1 (*e.g.* 2:1, 3:1, 4:1, 5:1, 10:1 or higher). Different saccharides can be conjugated to the same or different type of carrier protein. Any suitable conjugation reaction can be used, with any suitable linker where necessary.

Toxic protein antigens may be detoxified where necessary *e.g.* detoxification of pertussis toxin by chemical and/or genetic means.

Where a diphtheria antigen is included in the composition it is preferred also to include tetanus antigen and pertussis antigens. Similarly, where a tetanus antigen is included it is preferred also to include diphtheria and pertussis antigens. Similarly, where a pertussis antigen is included it is preferred also to include diphtheria and tetanus antigens.

Antigens in the composition will typically be present at a concentration of at least 1μg/ml each. In general, the concentration of any given antigen will be sufficient to elicit an immune response against that antigen.

As an alternative to using protein antigens in the composition of the invention, nucleic acid encoding the antigen may be used {*e.g.* refs. lxxxii to xc}. Protein components of the compositions of the invention may thus be replaced by nucleic acid (preferably DNA *e.g.* in the form of a plasmid) that encodes the protein.

Definitions

The term "comprising" means "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* means, for example, $x \pm 10\%$.

References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of reference xci. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is disclosed in reference xcii.

EXAMPLES

The present invention will be defined only by way of example in which reference is made to the following Figures. It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

Figure 1. Western blot analysis of total protein extracts from *C. trachomatis* EBs, performed using mouse immune sera against recombinant antigens. Only FACS positive non neutralizing sera are shown. For antigen identification, refer to Table 1. The panel identification numbers correspond to the numbers reported in the WB analysis column of Table 1. In each panel, the strip on the right shows the results obtained with the antigen-specific immune serum (I), and the strip on the left shows the results obtained with the corresponding preimmune serum (P).

Figure 2: Serum titres giving 50% neutralization of infectivity for the 9 *C. trachomatis* recombinant antigens described in the text. Each titre was assessed in 3 separate experiments (SEM values shown).

Figure 3: FACS analysis of antibody binding to whole *C. trachomatis* EBs. Gray histograms (event counts versus fluorescence channels) are the FACS output for EBs stained with background control antibodies. White histograms are the FACS output of EBs stained with antigen-specific antibodies. Positive control was represented by an anti-*C. trachomatis* mouse hyperimmune serum against whole EBs, with the corresponding preimmune mouse serum as background control; Negative controls were obtained by staining EBs with either mouse anti-GST or mouse anti-HIS hyperimmune serum, with the corresponding preimmune serum as background control. For each serum the background control was represented by mouse anti-GST or mouse anti-HIS hyperimmune serum, depending on the fusion protein used for immunization.Western blotting data obtained from total EB proteins stained with the same antiserum used for the FACS assays are also shown within each panel.

Figure 4 shows a Faster Clearance of *Chlamydia trachomatis* (CT) at 21 days post-challenge in mice vaccinated with a mixture of CT242 (OmpH-like) and CT316 (L7/L12) in combination with CFA.

Figure 5 shows a Faster Clearance of *Chlamydia trachomatis* (CT) at 21 days post-challenge in mice vaccinated with a mixture of CT467 (AtoS) and CT444 (OmcA) in combination with CFA.

Figure 6 shows a Faster Clearance of *Chlamydia trachomatis* (CT) at 21 days post-challenge in mice vaccinated with a mixture of CT812 (PmpD) and CT082 (Hypothetical) in combination with CFA.

Figures 7(a) and 7(b) show a stastically significant clearance of Chlamydia trachomatis at 14 days post-challenge in mice vaccinated with a mixture of CT242 and CT316 in combination with CFA.

Figure 7(c) shows the neutralization titre for mice vaccinated with a mixture of CT242 and CT316 in combination with CFA

Figures 8(a) and 8(b) show a clearance of Chlamydia trachomatis at 14 days post-challenge in mice vaccinated with a mixture of five CT antigens, these being CT 045, CT089, CT396, CT398 and CT381 in combination with AlOH and CpG.

Figure 8© shows the IgG antibody isotypes (IgG1 and IgG2) for pre-challenge sera from mice vaccinated with a mixture of five CT antigens, these being CT 045, CT089, CT396,CT398 and CT381 in combination with AlOH and CpG.

Figures 9(a) and 9(b) show a clearance of *Chlamydia trachomatis* (CT) at 7, 14 and 21 days post-challenge in mice vaccinated with a mixture of five CT antigens, these being CT 045, CT089, CT396, CT398 and CT381 in combination with AlOH and CpG.

Figure 9© shows the neutralization titre and IgG antibody isotypes (IgG1 and IgG2) for pre-challenge sera from mice vaccinated with a mixture of five CT antigens, these being CT 045, CT089, CT396, CT398 and CT381 in combination with AlOH and CpG.

Figures 10(a) and (b) show the neutralization titre for for mice vaccinated with a mixture of five CT antigens, these being CT 045, CT089, CT396, CT398 and CT381 in combination with AlOH and CpG.

Table 1(a): Characterisation of Expressed *Chlamydia trachomatis* (CT) Proteins

Gene identification number (Gene ID) and the corresponding annotation were retrieved from the D/UW-3/CX genome filed in GenBank (accession number AE001273).

Theoretical molecular masses (in kilodaltons) were calculated for predicted mature forms.

The Western blot column (WB profile) summarizes the results obtained by probing total EB proteins with antisera against recombinant proteins. The number in brackets refers to panel number in Figure 2. WB results are classified as follows: C, consistent (the predominant band observed is consistent with the expected molecular weight; additional minor bands may also be present); PC, partially consistent (a band of expected molecular weight is present together with additional bands of higher molecular weight or greater intensity); NC, nonconsistent (the detected bands do not correspond to the expected molecular weight); N, negative (no profile obtained).

FACS results are expressed as K-S scores. The serum titers giving 50% neutralization of infectivity for the 9 *C. trachomatis* recombinant antigens described in the text. Each titer was assessed in 3 separate experiments (SEM values shown). All of the proteins that showed a K-S score higher than 8.0 have been listed as FACS-positive.

Table 1(a): Characterisation of *Chlamydia trachomatis* (CT) expressed proteins

Gene ID	Protein ID	Current annotation	Fusion type	Theoretical MWt (kDa)	Antiserum: WB analysis	Antiserum: FACS assay (KS score)	Antiserum: Neutralizing titre (reciprocal)	Antigen: Reported 2DE / MALDI-TOF detection
CT045	PepA	*pep* A (Leucyl Aminopeptidase A)	HIS	54.0	C	16.81	100	Yes
CT381	ArtJ	artJ (Arginine Binding Protein)	HIS	26.0	C	32.54	370	No
CT396	DnaK	dnaK (HSP-70 heat shock protein)	HIS	70.6	C	34.50	230	Yes
CT398	CT398	Hypothetical protein	His&GST	29.4	C	31.24	540	Yes
CT547	CT547	Hypothetical protein	HIS	32.6	PC	28.21	40	No
CT587	Enolase	eno (Enolase)	HIS	45.3	C	20.85	180	Yes
CT681	MOMP	ompA (Major Outer Membrane Protein)	HIS	40.1	C	34.66	160	Yes
CT242	OmpH	ompH-Like Outer Membrane Protein	HIS	15.8	C	<8	190	No
CT467	AtoS	atoS (2-component sensor histidine kinase)	GST	39.8	N	<8	500	No
CT043	CT043	hypothetical <<Cpn0387	GST	18.4	?	27.53	?	?
CT050	CT050	Hypothetical protein	GST	56.6	C (1)	20.68	< 30	No
CT082	CT082	Hypothetical protein.	GST	59.4	C (2)	25.63	< 30	Yes
CT089	LcrE	lcrE (Low Calcium response E)	HIS	43.0	C (3)	12.59	< 30	No
CT128	Adk	adk (adenylate kinase)	GST	27.6	C (4)	16.00	< 30	No
CT153	CT153	hypothetical >Cpn0176 (6445) <<MAC/perforin domain	GST	90.8	?	13.33	?	?
CT157	CT157	Phospholipase D Superfamily	GST	45.2	C (5)	19.77	< 30	No
CT165	CT165	Hypothetical protein	GST	16.8	C (6)	10.46	< 30	No
CT262	CT262	hypothetical > Cpn0411	His-ib	28.7	?	19.31	?	?
CT266	CT266	Hypothetical protein >Cpn0415(6696)	HIS	43.9	PC (7)	21.29	< 30	No
CT276	CT276	hypothetical (acidic) > Cpn0425 (6706)	GST	21.3	?	19.85	?	?
CT296	dcrA	hypothetical divalent cation dependent regulator (Raulston)	GST	17.9	?	17.70	?	?
CT316	L7/L12	rl7 (Ribosomal protein L7/L12)	HIS	13.4	C (8)	9.68	< 30	Yes
CT372	CT372	hypothetical (basic)	His	49.3	?	24.77	?	?
CT443	OmcB	omcB (60kDa Cysteine-Rich OMP)	HIS	56.2	C (9)	21.28	< 30	Yes
CT444	OmcA	omcA (9kDa Cysteine-Rich OMP)	GST	9.0	PC (10)	15.00	< 30	No
CT456	CT456	Hypothetical protein	GST	97.6	N (11)	10.90	< 30	Yes
CT480	oppA	oligopeptide binding protein (1 of 5 genes)	pHis&pGST	58.8	?	27.45/9.48	?	?
CT541	Mip-like	mip (FKBP-type cis-trans isomerase)	GST	24.5	C (12)	9.94	< 30	Yes
CT548	CT548	hypothetical	GST	?	?	14.78	?	?
CT559	YscJ	yscJ (Yop proteins translocation lipoprotein J)	HIS	33.3	C (13)	23.21	< 30	No
CT600	Pal	pal (Peptidoglycan-Associated Lipoprotein)	HIS	19.1	C (14)	10.46	< 30	No
CT623	CT623	CHLPN 76kDa Homolog	GST	45.6	C (15)	15.89	< 30	No
CT635	CT635	hypothetical	His&GST	?	?	11.62/11.52	?	?
CT671	CT671	hypothetical	his	?	?	9.29	?	?
CT713	PorB	porB (Outer Membrane Protein Analog)	HIS	34.4	C (16)	25.82	< 30	Yes
CT823	HtrA	htrA (DO serine protease)	HIS	51.4	PC (17)	26.62	< 30	Yes
CT859	CT859	metalloprotease	his&GST	?	?	10.91/9.46	?	?
CT412	pmpA	OM protein A	His	105.6	?	10.92	?	?
CT414	PmpC	pmpC (Putative outer membrane protein C)	GST	184.9	C (18)	9.03	< 30	No
CT812	PmpD	pmpD (Putative Outer Membrane Protein D)	GST	157.6	N (19)	10.43	< 30	Yes
CT869	PmpE	pmpE (Putative Outer Membrane Protein E)	HIS	102.7	N (20)	15.28	< 30	No

Table 1(b): Characterisation of Expressed *Chlamydia trachomatis* (CT) Proteins cont
Gene identification number (Gene ID) and the corresponding annotation were retrieved from the D/UW-3/CX genome filed in GenBank (accession number AE001273).
Theoretical molecular masses (in kilodaltons) were calculated for predicted mature forms. FACS results are expressed as K-S scores.
The W-B analyses were carried out as described for Table 1 above.
FACS results are expressed as K-S scores as described for Table 1 above.
In-vitro neutralisation assays: neg = negative; ND = not determined

Table 1(b)

Gene ID	Gene Annotation	Fusion Type	Molecular Mass (kDa)	Western Blot (WB)	K-S Score	In –vitro neut activity
CT016	Hypothetical	HIS	26.63	Neg	17.94	neg
CT017	Hypothetical	HIS	47.79	Neg	12.18	neg
CT043	Hypothetical	HIS	18.38	Consistent	27.53	neg
CT082	Hypothetical	HIS	59	Partly C	15.89	neg
CT548	Hypothetical	GST	21.9	C	14.78	neg
CT153	Hypothetical	GST	90.86	C	13.33	neg
CT262	Hypothetical	HIS	28.81	Neg	19.31	neg
CT276	Hypothetical	GST	21.37	Not C	19.85	neg
CT296	Hypothetical	GST	17.98	Neg	17.70	neg
CT372	Hypothetical	HIS	49.00	Partly C	24.77	neg
CT398	Hypothetical	GST			27.03	neg
CT398	Hypothetical	HIS			22.96	neg
CT548	Hypothetical	GST			14.78	neg
CT043	Hypothetical	HIS			27.53	neg
CT635	Hypothetical	GST	16.77	Neg	11.52	ND
CT635	Hypothetical	HIS	16.77	Neg	11.62	ND
CT671	Hypothetical	HIS	31	Neg	20.91	ND
CT671	Hypothetical	GST	31	Neg	18.07	ND
CT089	Low Calcium Response Element (LcrE)	GST	44	C	11.9	neg
CT812	PmpD	GST	168	Not C	23.48	neg
CT412	Putative Outer Membrane Protein A	HIS	107	Not C	10.92	neg
CT480	Oligopeptide Binding Lipoprotein	GST	79.89	C	9.48	neg
CT480	Oligopeptide Binding Lipoprotein	HIS	79.89	C	27.45	neg
CT859	Metalloprotease	GST	34.21	C	9.46	ND
CT859	Metalloprotease	HIS	34.21	C	10.91	neg
CT869	PmpE	GST	106	PC	30.67	neg
CT053						ND

Example 1

MATERIALS AND METHODS

Preparation of *C. trachomatis* EBs and chromosomal DNA.

C. trachomatis GO/96, a clinical isolate of *C. trachomatis* serotype D from a patient with non-gonococcal urethritis at the Sant'Orsola Polyclinic, Bologna, Italy, was grown in LLC-MK2 cell cultures (ATCC CCL-7). EBs were harvested 48h after infection and purified by gradient centrifugation as described previously (22). Purified chlamydiae were resuspended in sucrose-phosphate transport buffer and stored at -80°C until use. When required, prior to storage EB infectivity was heat inactivated by 3 h of incubation at 56°C. Chromosomal DNA was prepared from gradient-purified EBs by lysing the cells overnight at 37°C with 10 mM Tris-HCl, 150 mM NaCl, 3 mM EDTA, 0.6% SDS, 100 μg of proteinase K/ml, sequential extraction with phenol, phenol-chloroform, and chloroform, alcool precipitation and resuspension in TE buffer, pH 8.

In silico analyses

All the 894 protein coding genes and the corresponding peptide sequences encoded by the *C. trachomatis* genome UW-3/Cx (23) were retrieved from the National Center for Biotechnology Information web site (http://www.ncbi.nlm.nih.gov/). Putative surface exposed proteins were selected primarily on the basis of GenBank annotation and sequence similarity to proteins known to be secreted or surface-associated. Sequences annotated as hypothetical, which typically lack significant homologies to well characterized proteins, were analyzed for the presence of leader peptide and/or transmembrane regions with PSORT algorithm (24). Following these criteria, a set of 158 peptides were selected for expression and in vitro screening.

Cloning and expression of recombinant proteins.

Selected ORFs from the *C.trachomatis* UW-3/Cx genome (23) were cloned into plasmid expression vectors so as to obtain two kinds of recombinant proteins: (i) proteins with a hexa-histidine tag at the C terminus (ct-His), and (ii) proteins fused with both glutathione *S*-transferase (GST) at their N terminus and a hexa-histidine tag at their C terminus (Gst-ct) as described (25). *Escherichia coli* BL21 and BL21(DE3) (Novagen) were the recipient of pET21b-derived recombinant plasmids and pGEX-derived plasmids respectively. PCR primers were designed so as to amplify genes without the signal peptide coding sequence. When a signal peptide or processing site was not clearly predictable, the ORF sequence was cloned in its full-length form. Recombinant clones were grown in Luria-Bertani medium (500 ml) containing 100 ug of ampicillin/ml and grown at 37°C until an optical density at 600 nm (OD600) of 0.5 was reached. Expression of recombinant proteins was then induced by adding 1 mM isopropyl-D-thiogalactopyranoside (IPTG). Three hours after IPTG induction, cells were collected by centrifugation at 6000 xg for 20 min. at 4 °C. Before protein purification, aliquots of the cell pellets (corresponding to an OD600 of 0.1) were resuspended in sample loading buffer (60 mMTris-HCl [pH 6.8], 5% [wt/vol] SDS, 10% [vol/vol] glycerol, 0.1% [wt/vol] bromophenol blue, 100 mM dithiothreitol [DTT]), boiled for 5 min, and analyzed bySDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Purification of recombinant proteins.

The cell pellets obtained from centrifugation of 500 ml induced recombinant *E. coli* cultures were suspended with 10 ml B-PER™ (Bacterial-Protein Extraction Reagent, Pierce), 1 mM MgCl2, 100 Kunits units DNAse I (Sigma), and 1 mg/ml lysozime (Sigma). After 30 min at room temperature under gentle shaking the lysate was clarified by centrifugation at 30.000 g for 30 min at 4 °C and the supernatant (soluble proteins) was separated from the pellet (debris, insoluble proteins and inclusion bodies).

Soluble His-tagged proteins were purified by an immobilized metal affinity chromatography (IMAC) using 1 ml mini-columns of Ni-activated Chelating Sepharose Fast Flow (Amersham). After loading the column was washed with 20 mM Imidazole and the remaining proteins were eluted by one step elution using 250 mM Imidazole buffer, 50 mM phosphate, 300 mM NaCl, pH 8.0.

Insoluble His-tagged proteins were purified by suspending the pellet, coming from centrifugation of B-PER lysate, in 50 mM TRIS-HCl, 1 mM TCEP {Tris(2-carboxyethyl)-phosphine hydrochloride, Pierce) and 6M guanidine hydrochloride, pH 8.5, and performing an IMAC in denaturing conditions of the clarified solubilized proteins. Briefly: the resuspended material was centrifuged at 30.000 g for 30 min and the supernatant was loaded on 1 ml minicolumns of Ni-activated Chelating Sepharose Fast Flow (Pharmacia) equilibrated with 50 mM TRIS-HCl, 1 mM TCEP, 6M guanidine hydrochloride, pH 8.5. The column was washed with 50 mM TRIS-HCl buffer, 1 mM TCEP, 6M urea, 20 mM imidazole, pH 8.5. Recombinant proteins were then eluted with the same buffer containing 250 mM imidazole.

The soluble GST-fusion proteins were purified by subjecting the B-PER soluble lysate to glutathione affinity purification using 0,5 ml mini-columns of Glutathione-Sepharose 4B resin (Amersham) equilibrated with 10 ml PBS, pH 7.4. After column washing with equilibrium buffer the proteins were eluted with 50 mM TRIS buffer, 10 mM reduced glutathione, pH 8.0. Protein concentration was determined using the Bradford method.

Eluted protein fractions were analyzed by SDS-Page and purified proteins were stored at –20 °C after addition of 2 mM Dithiothreitol (Sigma) and 40 % glycerol.

Preparation of mouse antisera

Groups of four 5- to 6-week-old CD1 female mice (Charles River, Como, Italy) were immunized intraperitoneally at days 1, 15, and 28 with 20 ug of purified recombinant protein in Freund's adjuvant. Pre-immune and immune sera were prepared from blood samples collected on days 0 and 43 respectively and pooled before use. In order to reduce the amount of antibodies possibly elicited by contaminating E.coli antigens, the immune sera were incubated overnight at 4°C with nitrocellulose strips adsorbed with an E. coli BL21 total protein extract.

Immunological assays.

For Western blot analysis (26), total proteins from purified *C. trachomatis* GO/96 serotype D EBs (2 ug per lane) were separated by SDS-PAGE (30) and electroblotted onto nitrocellulose membranes. After 30 min. of saturation with PBS-dried skimmed milk (5% w/v) membranes were incubated overnight with preimmune and immune sera (standard dilution 1:400) and then washed 3x with phosphate-buffered saline (PBS)-Tween 20 (0.1% v/v). Following a 1 hour incubation with a peroxidase-conjugated anti-mouse antibody (final dilution 1:5,000 Amersham;) and washing with PBS-Tween, blots were developed using an Opti-4CN Substrate Kit (Bio-Rad).

Flow cytometry assays

Analyses were performed essentially as previously described (25). Gradient purified,heat-inactivated GO/96 serotype D EBs (2x105 cells) from *C. trachomatis* resuspended in phosphate-saline buffer (PBS), 0.1% bovine serum albumin (BSA), were incubated for 30 min. at 4°C with the specific mouse antisera (standard dilution 1:400). After centrifugation and washing with 200

µl of PBS-0.1% BSA, the samples were incubated for 30 minutes at 4°C with Goat Anti-Mouse IgG, F(ab)'2-specific, conjugated with R-Phycoerythrin (Jackson Immunoresearch Laboratories Inc.). The samples were washed with PBS-0.1%BSA, resuspended in 150 µl of PBS-0.1%BSA and analysed by Flow Cytometry using a FACSCalibur apparatus (Becton Dickinson, Mountain View, CA). Control samples were similarly prepared. Positive control antibodies were: i), a commercial anti-*C. pneumoniae* specific monoclonal antibody (Argene Biosoft, Varilhes, France) and, ii), a mouse polyclonal serum prepared by immunizing mice with gradient purified *C. trachomatis* EBs.

Background control sera were obtained from mice immunized with the purified GST or HIS peptide used in the fusion constructs (GST control, HIS control). FACS data were analysed using the Cell Quest Software (Becton Dickinson, Mountain View, CA). The significance of the FACS assay data has been elaborated by calculating the Kolmogorov-Smirnov statistic (K-S score.) (44)([Young, 1977 #5718]). The K-S statistic allows determining the significance of the difference between two overlaid histograms representing the FACS profiles of a testing protein antiserum and its relative control. All the proteins that showed a K-S score higher than 8.0 have been listed as FACS positive, being the difference between the two histograms statistically significant ($p<0.05$). The D/s(n) values (an index of dissimilarity between the two curves) are reported as "K-S score" in Table 1.

In vitro neutralization assays

In vitro neutralization assays were performed on LLC-MK2 (Rhesus monkey kidney) epithelial cell cultures. Serial four-fold dilutions of mouse immune and corresponding preimmune sera were prepared in sucrose–phosphate–glutamic acid buffer (SPG). Mouse polyclonal sera to whole EBs were used as positive control of neutralization, whereas SPG buffer alone was used as negative control of neutralization (control of infection). Purified infectious EBs from *C. trachomatis* GO/96 serotype D were diluted in SPG buffer to contain $3x10^5$ IFU/ml, and 10 □l of EBs suspension were added to each serum dilution in a final volume of 100□l. Antibody-EB interaction was allowed to proceed for 30 min at 37°C on a slowly rocking platform. The 100□l of reaction mix from each sample was used to inoculate PBS-washed LLC-MK2 confluent monolayers (in triplicate for each serum dilution), in a 96-well tissue culture plate, and centrifuged at 805 x g for 1 hour at 37°C. After centrifugation Eagle's minimal essential medium containing Earle's salts, 20% fetal bovine serum and 1 ug/ml cycloheximide was added. Infected cultures were incubated at 37°C in 5%CO_2 for 72 hours. The monolayers were fixed with methanol and the chlamydial inclusions were detected by staining with a mouse anti-Chlamydia fluorescein-conjugated monoclonal antibody (Merifluor Chlamydia, Meridian Diagnostics, Inc.) and quantified by counting 5 fields per well at a magnification of 40X. The inhibition of infectivity due to EBs interaction with the immune sera was calculated as percentage reduction in mean IFU number as compared to the SPG (buffer only)/EBs control. In this calculation the IFU counts obtained with immune sera were corrected for background inhibition of infection due to the corresponding pre-immune mouse serum. According to common practice, the sera were considered as "neutralizing" if they could cause a 50% or greater reduction in infectivity. The corresponding neutralizing titer was defined as the serum dilution at which a 50% reduction of infectivity was observed. Experimental variability was evaluated by calculating the standard error of measurement (SEM), from three titration experiments for each recombinant antigen, as shown in Fig.2.

RESULTS 1

In silico selection.

The genomic ORFs to be expressed and submitted to functional screenings were selected on the basis of *in silico* analyses and literature searches, using bioinformatics tools and criteria similar to those described in a previous similar study on *C. pneumoniae* (Montigiani, et al., 2002). Essentially, we searched the genome of *C. trachomatis* serovar D for ORF's encoding proteins likely to be located on the surface of EBs. In order to maximize the chances of identifying bacterial surface proteins we initially selected *C.trachomatis* proteins having a significant sequence similarity to proteins found to be surface exposed in *C. pneumoniae* as previously reported (Montigiani, et al., 2002). A second step search was based essentially on the presence of a recognizable leader peptide (mostly as detected by the PSORT software), predicted transmembrane regions, and/or remote sequence similarities to surface proteins of other gram-negative bacteria detected with PSI-Blast runs against the non-redundant GenBank protein database. A third criterion was the addition to the panel of proteins described as immunogenic in animal models and humans. Using this procedure we selected a total of 158 ORFs, 114 of which had at least 40% of identity to proteins of *C.pneumoniae*, while 44 remained below such threshold and were considered as *C.trachomatis* specific.

Antigen cloning and expression. The 158 ORFs were amplified by PCRs and cloned in two different E. coli expression vectors in order to obtain each antigen as GST and/or His-tag fusion protein. Considering that the presence of an N-terminal signal peptide could have induced a possible targeting of the recombinat protein toward the *E. coli* cytoplasmic membrane, the N-terminal signal peptide nucleotide sequence was excluded from the expression construct. By the analysis of the ORFs expression we found that 94% of the selected genes could be expressed and 87% of them (corresponding to 137 different ORFs) could also be purified to recombinant fusion proteins that could be used as antigens for mice immunization. In total, 259 recombinant *C. trachomatis* fusion proteins, deriving from the 137 different genes cloned, were obtained and analysed for their quality in order to be used as antigens for mice immunization. Mice were immunized with 201 recombinant *C.trachomatis* fusion proteins to produce mouse sera that have been analysed for their capability to recognize surface exposed proteins on *C.trachomatis* EBs and their capability of interfering with the process of in vitro infection of epithelial cell culture.

Identification of surface exposed proteins by flow cytometry. Mice were immunized with 201 recombinant *C.trachomatis* fusion proteins to produce mouse sera that have been analysed both for their capability to recognize surface exposed proteins on *C.trachomatis* EBs and their capability of interfering with the process of *in vitro* infection of epithelial cell culture. Immunofluorescent staining of *C. trachomatis* EBs and flow cytometric analysis have been used to investigate the capability of mouse sera, obtained by immunization with a panel of 137 different *C. trachomatis* recombinat antigens, to recognize possibly surface exposed proteins. We had previously shown that flow cytometry can be a very useful tool to detect antibody binding to the surface of chlamydial EBs, by identifying a new panel of *C. pneumoniae* surface exposed proteins. Although *C. trachomatis* serovar L and E had already been analyzed by flow cytometry (Waldman, et al., 1987), (Taraktchoglou, et al., 2001), we first verified if this method could also be applied to *C. trachomatis* serovar D EBs analysis, by setting up a series of positive and negative controls. As shown in Fig 3, Panel A, a mouse polyclonal serum obtained by immunizing mice with purified whole *C. trachomatis* serovar D EBs, can significantly shift the flow cytometric profile of the bacterial cell population, as compared to a negative, pre-immune serum. As a positive control we also used a commercial anti-MOMP *C. trachomatis* specific monoclonal antibody (Argene), which gave a similar result as the polyclonal serum (data not shown). We also set up a series of negative controls, to exclude possible cross-reactions between

mouse sera and the chlamydial cell surface. In particular sera obtained by immunizing mice with the protein fraction eluted from the Ni columns loaded with a BL21(pET21b+) protein extract (His control, Fig.3, Panel 2) and with GST protein (GST control, Fig.3, Panel 3) were compared to the respective pre-immune sera. Negative controls never showed a shift of the histogram as compared to pre-immune sera. The control results indicated the specificity and reliability of the flow cytometric assay we set up.

We then analyzed all sera raised against recombinant *C.trachomatis* antigens for their capability to recognize surface exposed proteins on purified EBs, as determined by FACS binding assay. All the proteins that showed a K-S score higher than 8.0 have been listed as FACS positive, being the difference between the testing and the control histograms statistically significant ($p<0.05$). Of 137 different gene products analyzed, 28 showed to be able to induce antibodies capable of binding to the surface of purified EBs. Proteins that showed a positive result have been listed in Tables 1(a) and 1(b). The protein list in Table 1(a) is divided into two sections: (i) proteins that gave a positive result in the FACS assay and/or in the neutralization assay, therefore considered to be possibly surface exposed and with a neutralizing effect; (ii) proteins that showed to be able to induce antibodies directed versus surface exposed proteins of the EBs but did not show a detectable neutralizing effect. A comparative analysis of the proteins that resulted to be surface exposed in the *C. trachomatis* genomic screening shows that 21 out of 28 FACS positive antigens have a degree of homology higher than 40% to *C. pneumoniae* proteins that, as published in our previous work (Montigiani, et al., 2002), are likely surface exposed.

Analysis of the antisera to the recombinant antigens by Western blotting.

The panel of sera was also screened by Western blot analysis on whole protein extracts of purified chlamydial EBs, in order to visualize their capability to recognize a band of the expected molecular weight. The results of this analysis are reported in Tables 1(a) and 1(b), while the Western blot profiles are shown in Figures 1. In total, 22 out of the 30 sera described in Table 1(a) resulted to be "consistent", that is they appeared to recognize a band of the expected molecular weight on EBs protein extracts. Four sera, (anti-CT547, anti-CT266, anti-CT444, anti-CT823) were classified as "partially consistent", due to the presence of a band at the expected molecular mass plus few different bands of weaker intensity. Finally, four sera gave a negative Western blot pattern (anti-CT467, anti-CT456, anti-CT812, anti-CT823). Three out of the four Western blot negative sera (anti-CT456, anti-CT812, anti-CT823) gave a positive result in the FACS binding assay, even if with not very high K-S scores (K-S<15). It is worth noting that two of the Western blot negative sera were raised against antigens (CT812, CT823) belonging to the Pmp family (PmpD and PmpG), a Chlamydia specific family of complex proteins many of which have already been localized on the chlamydial cell surface at least in *C.pneumoniae* (Knudsen, et al., 1999) (Christiansen, et al., 1999) (Mygind, et al., 2000; Vandahl, et al., 2002)) (Montigiani, et al., 2002) . The Western blot negative serum obtained by immunization with CT467 (AtoS) was scored as negative also in the FACS assay, but surprisingly it showed a high neutralizing titer (Fig.2).

Evaluation of the antisera for *in vitro* neutralizing properties.

An *in vitro* neutralization assay on purified *C. trachomatis* EBs allowed us to identify neutralizing antigens. Infectious EBs were pre-incubated with the mouse antisera obtained with *C. trachomatis* recombinant antigens and then tested for their capability to infect a monolayer of epithelial cells. By using this assay, as summarized in Table 1 (a)(section 1) 9 sera have proved to be effectively neutralizing at a dilution higher than 1:30. These 9 sera were obtained by immunizing mice with recombinant proteins encoded by the following *C.trachomatis* genes:

pepA(CT045), encoding a leucyl aminopeptidase; *artJ(CT381)*, encoding a putative extracellular solute (possibly Arginine) binding protein of an aminoacid transport system; *dnaK(CT396)*, encoding a well described chaperonin of the hsp70 family; two "hypothetical" genes CT398 and CT547; *eno(CT587)*, encoding a protein homologous to bacterial enolases, glycolytic enzymes that can be found also on bacterial surfaces; *ompA(CT681)*, encoding the major outer membrane protein; CT242 (OmpH-like), encoding a protein homologue to of the OmpH family of bacterial proteins, some members of which have been reported to be chaperones involved in outer membrane byosinthesis; *atoS (CT467)*, encoding a putative sensor member of a transport system. As shown in figure 2, and summarized in Table 1, three of the recombinant antigens (ArtJ (CT381), CT398 and AtoS (CT467)) were able to induce antibodies with high neutralizing activity (neutralizing serum titers above 1:300); four of them (DnaK (CT396), Enolase (CT587), OmpA (and OmpH-like (CT242)) induced sera with intermediate neutralizing titers (between 1:180 and 1:300), finally sera raised against two proteins (PepA (CT045) and CT547) had titers equal or less than 100. Figure3, on Panels 4 to 12, shows the FACS profiles of the 9 proteins that resulted to be neutralizing, demonstrating that 7 of them are able to induce antibodies directed versus the surface of EBs, while two of them (OmpH-like and AtoS) did not show this capability. The Western blot profiles, against whole-EBs protein extracts, of the sera raised against the FACS-positive neutralizing antigens (Fig. 3) resulted to be either fully consistent, i.e. with a single band of the expected molecular weight (CT045-PepA, CT381-ArtJ) or partially consistent , i.e. showing a major band of the expected molecular weight besides other bands (CT396-DnaK, CT398, CT547, CT587-Enolase, CT681-MOMP). However, in the case of CT396 (DnaK) and CT681 (MOMP), it should be noted that previous work using 2D electrophoretic mapping and either immunoblotting with a specific monoclonal (Bini, et al., 1996)) or spot identification by mass spectrometry (Shaw, et al., 2002)) shows that these proteins do appear in EB extracts as multiple electrophoretic species of different Mw, probably due to processing and/or post-translational modifications. Of the 3 remaining 'partially consistent' profiles, those obtained with the antisera to recombinant CT398 and CT547-Enolase show that the antibodies recognize predominantly a band of the expected size, whereas in the case of the hypothetical CT547 there is in fact a doubt about the specificity of the antiserum. The two FACS negative and neutralizing antigens showed a different behavior. While the Western blot profile of CT242 (OmpH-like) is fully consistent showing a single band of the expected molecular weight (Fig.3, Panel 8), the blot of CT467 (AtoS) resulted to be completely negative (Fig.3, Panel 9).

In the case of the anti-OmpH (CT242) serum the apparent contradiction between FACS and Western blot profiles could be explained assuming a different sensitivity between the two assays. However, the AtoS (CT467) results remain contradictory. Considering that the above findings could be partially explained by the fact that for safety reasons the FACS analyses were performed on heath-inactivated preparations of EB and that the inactivation procedure could have totally (anti-AtoS) or partially (anti-OmpH) destroyed conformational epitopes essential for antibody binding, we also tested these antisera in a dot-blot assay (REF) using infectious EBs spotted on a nitrocellulose membrane, as described by Kawa and Stephens (Kawa and Stephens, 2002). However, the dot-blot assay results only confirmed the results obtained with the FACS assay.

Discussion of Results 1

Tables 1(a) and 1(b) present the results of FACS and the '*in vitro* neutralization' assays obtained from sera raised against a set of *C.trachomatis* recombinant fusion proteins, of which, so far, 9 "neutralizing" antigens were identified. With the exception of MOMP (Caldwell and Perry, 1982, Peterson et al., 1991, Su and Caldwell, 1991, Zhong et al., 1994, Fan and Stephens, 1997), none of these antigens has been previously reported as neutralizing. Previous literature also describes PorB (CT713) as a second neutralizing protein (Kawa and Stephens, 2002, Kubo and Stephens, 2000). However, as shown in Table 1(a), the serum against our recombinant form of PorB failed to neutralize *Chlamydia* infection *in vitro*. This discrepancy may be explained considering that our recombinant antigen was water-insoluble and therefore it might have lost the correct conformation required to induce neutralizing antibodies. The possibility of a similar situation should be kept in mind also in the interpretation of data relative to the other 'insoluble' antigens. It is interesting to note that, besides MOMP, other proteins in this selection, including PepA, DnaK, HtrA and PorB, have been reported as proteins which are immunogenic in the course of genital tract infection in humans (Sanchez-Campillo et al., 1999).

Apart from the CT antigens for which no in-vitro neutralizing data was available (CT635, CT671 and CT859 – marked as ND in Table 1(b)), none of the other CT specific proteins disclosed in Table 1(b) demonstrated in-vitro neutralizing activity. However, these in-vitro results do not mean or suggest that these CT specific antigens do not or may/could not demonstrate an in-vivo protective effect especially when used in combination with one or more other CT antigens with, for example, a complementary immunological profile (see for example, the protective effect against CT challenge which was obtained when combinations of CT antigens, such as (CT242 and CT316) and (CT467 and CT444) and (CT812 and CT082) with complementary immunological profiles are used.

Example 2

Table 1(b) also provides the FACS results obtained from sera raised against a set of 17 Chlamydia trachomatis recombinant fusion proteins, these being: CT016, CT017, CT043, CT082, CT153, CT262, CT276, CT296, CT372, CT398, CT548, CT043, CT635, CT671 (all Hypoythetical Proteins). CT412 (Putative Outer Membrane Protein), CT 480 (Oligopeptide Binding Protein), CT859 (Metalloprotease), CT089 (Low Calcium Response Element – LcrE), CT812 (PmpD) and CT869 (PmpE). FACS analysis was carried out on either the HIS fusion and/or the GST fusion. All of these CT recombinant fusion proteins showed a K-S score higher than 8.0 and were deemed FACS positive. With the exception of CT398, at least none of these Hypothetical proteins have been previously reported as FACS positive. These Hypothetical CT antigens are generally regarded are CT specific antigens and do not have a *C. pneumoniae* counterpart.

Example 3

Methodology

Mouse Model for in-vivo screening for CT protective antigens

A Mouse Model of *Chlamydia trachomatis* (CT) genital infection for determining in-vivo protective effect of CT antigens (resolution of a primary Chlamydia infection) was used. The model used is described as follows:

Balb/c female mice 4-6 weeks old were used;

The mice were immunized intra-peritoneally (ip) with a mixture of two recombinant CT antigens in the groups as set out in Table 2 below. These CT antigens were determined to be FACS positive and/or neutralizing (see Table 1(a)). Three doses of the CT antigen mixture was given.

The CT antigens in Groups 1 and 2 were HIS fusion proteins. The CT antigens used in Group 3-6 were GST fusion proteins. The mice were given hormonal treatment 5 days prior to challenge with 2.5mg of DepoProvera (medroxyprogesterone acetate).

Table 2

Group	*Immunising Composition*	*Immunoregulatory agent*	*Route of Delivery*
1	CT242 (OmpH-like) +CT316 (L7/L12) (20ug of each protein)	CFA	Intra-peritoneal (i.p,)
2	CT242+CT316 (20ug of each protein)	AlOH (200ug) + CpG (10ug)	Intra-peritoneal (i.p,)
3	CT467 (AtoS) +CT444 (OmcA) (20ug of each protein)	CFA	Intra-peritoneal (i.p,)
4	CT467+CT444 (20ug of each protein	AlOH (200ug) + CpG (10ug)	Intra-peritoneal (i.p,)
5	CT812 (PmpD)+CT082 (Hypothetical) (20ug of each protein)	CFA	Intra-peritoneal (i.p,)
6	CT812+CT082 (20ug of each protein)	AlOH (200ug) + CpG (10ug)	Intra-peritoneal (i.p,)
7 (Negative Control)	CFA		Intra-peritoneal (i.p,)
8 (Negative Control)	AlOH (200ug) + CpG (10ug)		Intra-peritoneal (i.p,)
9 (Positive Control)	Live Chlamyida EB		Intra-peritoneal (i.p,)

Test Challenges

The mice were challenged intravaginally with 10^5 IFU of purified EBs (Serovar D), 2 weeks after the last immunization dose. A read out of vaginal swabs every 7 days up to 28 days after challenge. The following assays were also carried out on pre-challenge sera: Serological analysis: FACS, WB, Neutralization assay and ELISA. The ELISA were performed by coating plates with each recombinant antigen and testing the reaction of immune sera from single mice immunized with the combination of two CT antigens. The data is expressed as the mean value calculated for each group expressed as mean ELISA units. The antibody type (IgG, IgA etc) and isotype was checked in serum post immunization but pre-challenge. The purpose of the serum studies was to determine how the mice responded to immunization with the CT antigen combinations. The purpose of the vaginal washes was to determine how the mice responded to the bacterial challenge. Antibody analyses in terms of antibody type (IgG and IgA) and antibody subtype were also carried out on the vaginal washes.

Negative Controls

The negative control used was the immunoregulatory agent alone (eg CFA or AlOH and/or CpG)

Positive "live" EB controls

The positive control used was an extract from live Chlamydia Elementary Bodies (EBs). Here the mice were infected with live *Chlamydia* EB at the same time that the test CT combination antigens were being administered. The "live" EB positive control animals were infected for about 1.5 months (ie 6 weeks) (because 3 doses of CT antigenic combinations were administered every 2 weeks (ie over a total of 6 weeks). The animals (mice) infected with "live" EB developed a natural immunity which resolved the infection (because *Chlamyida* infection in mice is a transient infection). When the mice vaccinated with the CT antigenic combinations were then challenged with "live" EB, the positive control "live" EB mice were also re-challenged (ie they were given a second dose of "live" EB). As the "live" EB positive control group developed a natural immunity, they cleared the second re-challenge quickly.

Results for 3 x 2CT antigenic combinations + CFA

Table 2 above shows the three combinations of two different CT antigens with complementary immunological profiles which are capable of providing protection against CT challenge in a mouse model of *Chlamydial* genital infection. The antigen combinations were administered in combination with either CFA or AlOH and CpG.

Figures 4-6

In the Figures 4-6 provided, the x axis denotes weeks post-challenge
The y axis denotes *Chlamydia trachomatis* units in terms of IFU /vaginal swab.
The results are expressed as mean of IFU/swab recovered for each group of mice
1= 1 week or day 7
2= 2 weeks or day 14
3 = 3 weeks or day 21
In each graph, both positive and negative control results are reported.
A negative control = mice immunized with adjuvant alone
A positive control = mice infected with 10 (to the power of 6) Chlamydia EB IFU and rechallenged (natural protection)
Results demonstrate that a protective effect for all 3 combinations of two CT antigen was observed at 21 days post challenge.

Figures 7(a), 7(b) and 7(c)

The vacciniation protocol for mice in Group 1 of Table 2 was repeated and the results obtained are set out in Figures 7(a)-(c).
Figures 7(a) and 7(b) demonstrate a significant protection 14 days after CT challenge in mice immunized with a combination of CT242 and CT316 antigens and CFA adjuvant. In Figures 7(a) and 7(b) it is clear that at 7 days post challenge, only 50IFU *Chlamydia* are present in the live controls compared with about 100 fold more *Chlamydia* (it about 5000IFU) in the test mice. However, at 14 days post-challenge, the vaccinated mice have cleared the Chlamydia infection to almost the same level as the "live" EB positive control mice indicating the mice vaccinated with a combination of CT242 and CT316 + CFA have almost the same level of protective immunity as the "natural" immunity developed by the "live" EB control mice.
Figure 7© indicates that the serum dilution at which a 50% reduction in infection was observed was 1:50 indicating the presence of in-vitro neutralizing activity.

Results 3 Discussion

Figures 4-6 and Figure 7(a)-(c) demonstrate that three combinations of two different CT antigent with complementary immunological profiles are capable of providing protection against CT challenge in a mouse model of Chlamydial genital infection when administerd in combination with an immunoregulatory agent.

Example 4: Immunizations with Combinations of the First Antigen Group

The five antigens of the first antigen group ((OmpH-like protein, ArtJ, DnaK, CT398 and HrtA) or other combinations of CT antigens as already described) were prepared as described in reference xvii. The antigens are expressed and purified. Compositions of antigen combinations are then prepared comprising five antigens per composition (and containing 15 μg of each antigen per composition).

CD1 mice are divided into seven groups (5-6 mice per group for groups 1 through 6; 3 to 4 mice for groups 5, 6, 7, 8 and 9), and immunized as follows:

Table 3

Group	Immunizing Composition	Route of Delivery
1	Mixture of 5 antigens (15 μg/each) + CFA	Intra-peritoneal or intra-nasal
2	Mixture of 5 antigens (15 μg/each) +AlOH (200μg)	Intra-peritoneal or intra-nasal
3	Mixture of 5 antigens (15 μg/each) +CpG (10ug)	Intra-peritoneal or intra-nasal
4	Mixture of 5 antigens (15 μg/each) + AlOH (200μg) + CpG (10μg)	Intra-peritoneal or intra-nasal
5	Complete Freunds Adjuvant (CFA)	Intra-peritoneal or intra-nasal
6	Mixture of 5 antigens (5 μg/each) + LTK63 (5μg)	Intra-peritoneal or Intranasal
7	AlOH (200μg) + CpG (10μg)	Intra-peritoneal or intra-nasal
8	CpG (10μg)	Intra-peritoneal or intra-nasal
9	LTK63 (5μg)	Intra-peritoneal or intra-nasal

Mice are immunized at two week intervals. Two weeks after the last immunization, all mice are challenged by intravaginal infection with *Chlamydia trachomatis* serovar D.

Example 5

Mouse Model for in-vivo screening for CT protective antigens

A Mouse Model of *Chlamydia trachomatis* genital infection for determining in-vivo protective effect of CT antigens (resolution of a primary *Chlamydia* infection) was used. The model used is described as follows: Balb/c female mice 4-6 weeks old were used;

The mice were immunized intra-peritoneally (ip) with a mixture of five recombinant CT antigens as set out in Table 4 below. These CT antigens were determined to be FACS positive and/or neutralizing (see Table 1(a)). Three doses of the CT five antigen mixture were given at a concentration of 15ug per dose. The CT antigens listed in Groups 1 –3 of Table 4 were HIS

fusion proteins. The mice were given hormonal treatment 5 days prior to challenge with 2.5mg of DepoProvera (medroxyprogesterone acetate).

Table 4

Group	Immunising Composition	ImmunoRegulatory Agent	Route of Delivery
1 (Test Group)	CT045 + CT089 + CT396 + CT398 + CT381 (15ug/each CT antigen)	CFA	Intra-peritoneal (i.p.)
2 (Test Group)	CT045 + CT089 + CT396 + CT398 + CT381 (15ug/each CT antigen)	AlOH (200ug) and CpG (10ug)	Intra-peritoneal (i.p.)
3 (Test Group)	CT045 + CT089 + CT396 + CT398 + CT381 (15ug/each CT antigen)	AlOH (200ug) alone	Intra-peritoneal (i.p.)
4 (Test Group)	CT045 + CT089 + CT396 + CT398 + CT381 (15ug/each CT antigen)	CpG (10ug) alone	Intra-peritoneal (i.p.)
5 (Negative control)	Complete Freunds Adjuvant (CFA) alone		Intra-peritoneal (i.p.)
6 (Negative Control)	AlOH (200μg) + CpG (10μg)		Intra-peritoneal (i.p.)
7 (Positive Control)	Live Elementary Body (EB) from Chlamydia		Intra-peritoneal (i.p.)

Test Challenges

The mice were challenged intravaginally with 10^5 IFU of purified EBs (Serovar D), 2 weeks after the last immunization dose. A read out of vaginal swabs every 7 days up to 28 days after challenge. The following assays were also carried out on pre-challenge sera: Serological analysis: FACS, WB, Neutralization assay and ELISA. The ELISA were performed by coating plates with each recombinant antigen and testing the reaction of immune sera from single mice immunized with the combination of five CT antigens. The data is expressed as the mean value calculated for each group expressed as mean ELISA units. The antibody type (IgG, IgA etc) and isotype was checked in serum post immunization but pre-challenge. The purpose of the serum studies was to determine how the mice responded to immunization with the CT antigen combinations. The purpose of the vaginal washes was to determine how the mice responsed to the bacterial challenge. Antibody analyses in terms of antibody type (IgG and IgA) and antibody subtype were also carried out on the vaginal washes.

Negative Controls

The negative control used was the immunoregulatory agent alone (eg CFA or AlOH and/or CpG).

Positive "live" EB controls

The positive control used was an extract from live Chlamydia Elementary Bodies (EBs). Here the mice were infected with live Chlamydia EB at the same time that the test CT combination antigens are being administered. The "live" EB positive control animals were infected for about 1.5 months (ie 6 weeks) (because 3 doses of CT antigenic combinations were administered every 2 weeks (ie over a total of 6 weeks). The animals (mice) infected with "live" EB developed a natural immunity and resolved the infection (because Chlamyida infection in mice is a transient infection). When the mice were vaccinated with the CT antigenic combinationse were then challenged with "live" EB, the positive control "live" EB mice were also re-challenged (ie they

were given a second dose of "live" EB). As the "live" EB positive control group developed a natural immunity, they cleared the second re-challenge quickly.

Results for 1 x5 combos + CFA

Figures 8(a)-8(c) show the results obtained after administration of a combination of five different CT antigens (CT045, CT089, CT396, CT398 and CT381) with complementary immunological profiles which demonstrate that this five antigen mix is capable of providing protection against CT challenge in a mouse model of *Chlamydial* genital infection when used in combination with an immunoregulatory agent.

Figure 8(a), 8(b) and 8(c)

In more detail:

Figure 8(b) provided, the x axis denotes results for day 14 post-challenge

The y axis denotes *Chlamydia trachomatis* challenge units in terms of IFU /swab at day 14.

The results are expressed as mean of IFU/swab recovered for each group of mice

Both positive and negative control results are reported.

A negative control = mice immunized with adjuvant alone

A positive control = mice infected with 10 (to the power of 6) Chlamydia EB IFU and rechallenged (natural protection)

The results demonstrate that a protective effect for a combinations of five CT antigens (CT045, CT089, CT396, CT398 and CT381) when used in combination with AlOH and CpG was observed at 14 days post challenge.

Figure 8© demonstrates that IgG1 and IgG2 antibody isotypes could be measured in mice serum obtained post-immunisation but pre-challenge. These IgG isotype profiles are indicative of a Th1 and a Th2 protective immune response. However, a higher level of IgG1 to IgG2 was obtained both for CFA and AlOH and CpG immunoregulatory agents with the highest IgG1 levels being obtained after administration of the 5 CT antigen mix in combination with AlOH and CpG.

Figures 9(a), 9(b) and 9(c)

The vaccination protocol for mice in Group 1 of Table 4 was repeated and the results obtained are set out in Figures 9(a)-(c). However, this time, only AlOH and CpG adjuvant was used.

Figures 9(a) and 9(b) demonstrate a significant protection at both 7 days and 14 days after CT challenge in mice immunized with a combination of the five CT antigens (CT045, CT089, CT396, CT398 and CT381) and AlOH and CpG adjuvant. In Figures 9(b) it is clear that at 7 days and 14 days post challenge, the vaccinated mice have cleared the *Chlamydial* infection to almost the same level as the "live" EB positive control mice indicating that mice vaccinated with a combination of five CT antigens (CT045, CT089, CT396, CT398 and CT381) and AlOH and CpG adjuvant had almost the same level of protective immunity as the "natural" immunity developed by the "live" EB control mice.

Figure 9© demonstrates that IgG1 and IgG2 antibody isotypes could be measured in mice serum obtained post-immunisation but pre-challenge. These IgG isotype profiles are indicative of a Th1 and a Th2 protective immune response. Figure 9© also indicates that the serum dilution at which a 50% reduction of *Chlamydial* infectivity was obtained was 1:120.

Neutralisation Data for the 5 Antigen Mix

Figures 10(a) and 10(b) indicate that neutralizing antibody levels obtained for the 5 CT mixture when combined with AlOH and CpG were approximately the same as those obtained for the "live" EB postive control groups. In this regard, the serum dilutions at which a 50% reduction of *Chlamydial* infectivity was obtained were 1:120 and 1:110 respectively.

Results 5 Discussion

Figures 8-10 demonstrate that combinations of five different CT antigent with complementary immunological profiles when used in combination with an immunoregulatory agent are capable of providing protection against CT challenge in a mouse model of *Chlamydial* genital infection

OVERALL DISCUSSION

According to a genomic strategy aiming at the identification of new vaccine candidates, which gave promising results for other bacterial pathogens, we expressed in *E.coli*, as recombinant fusion proteins, 158 ORFs selected *in silico* from the *C.trachomatis* genome, and likely to encode peripherally located proteins. Polyclonal antibodies to these proteins were raised in mice and assessed, in parallel screenings, (i), for their capacity to bind purified *Chlamydiae* in a flow cytometry assay (identifying FACS-positive sera and corresponding antigens), and, (ii), for their capacity to induce a >50% inhibition of *Chlamydial* infectivity for *in vitro* cell cultures (neutralizing sera and antigens). The specificity of the antisera, which were partially purified by adsorption on *E.coli* protein extracts, was assessed by Western Blot analysis of the sera diluted 1:400 (the same dilution found optimal for the FACS assay screening) which were tested against protein extracts of gradient-purified elementary bodies of *C.trachomatis*. The Western Blot results showed that the majority of the 30 FACS positive and/or neutralizing antisera recognized either a single protein band of expected molecular size, or that a band consistent with the expected chamydial antigen was anyway predominant in the WB profile, with only minor bands of different size. In fact only for 5 antigens a doubt remained as to the true specificity of the antiserum, namely in the case of the CT547 protein, for which the expected band was present but not predominant, and the 4 cases for which the WB obtained was completely blank (CT456, CT476-AtoS, and the two fusion proteins for pmpD (CT812) and pmpE (CT869).

The parallel screenings identified FACS-positive sera and corresponding antigens, and, so far, 9 'neutralizing' antisera and antigens (Table1(a)). Seven of these (the recombinant forms of PepA (CT045), ArtJ (CT381), DnaK (CT396), Enolase (CT587); the 2 hypothetical products of CT398 and CT547, and the well studied product of ompA better known as the Major Outer Membrane Protein, MOMP (CT681), of *C.trachomatis*) were both FACS-positive and neutralizing in vitro: the neutralization data therefore therefore seem to confirm that the binding observed in the FACS assay occurred to intact infectious EBs. On the contrary, the two recombinant antigens obtained for the OmpH-like (CT242) and AtoS (CT467) proteins elicited antibodies with in vitro neutralizing properties, but surprisingly failed to show any measurable binding in the FACS assay (Fig.2 and 3). The results obtained for CT242 and CT467 are surprising and unexpected as these antigens appear not to be surface-exposed and yet both have high in-vitro neutralizing titres.

AtoS (CT467)

AtoS is a particular case in that the antiserum failed to detect any protein species by Western Blot analysis, and gave negative FACS assay results (with a K-S score below cut-off threshold). Nevertheless this antiserum yielded one of the best neutralization titres, second only to that elicited by the CT398 'hypothetical' protein. Interestingly, in the previous similar screening on *Chlamydia pneumoniae* (Cpn) antigens (Montigiani et al (2002) Infect Immun 70: 368-379), the antiserum to the homolog Cpn-AtoS proved again to be WB negative, but in this case FACS positive (KS=14.61) and capable of neutralizing (average titre=270) Cpn in vitro infection of the same cell line used in the present study. The apparent inconsistency of these results may be explained by considering that an antigen present in very small amounts in the EB sample could

bind too little antibody to be detected in the FACS binding assay, however it could become detectable by the in vitro neutralization assay owing to the possibility of using higher concentration of antibodies and to the amplification provided by the chlamydial replication in this type of assay. The hypothesis that AtoS is somehow lost in purified EBs, e.g. due to a particular instability, is in agreement with the fact that the AtoS protein, shown to be the sensor moiety of a 2-component system composed by AtoS and AtoC was never observed so far by mass spectrometry analysis of 2DE proteomic map nor in any of 3 CT serotypes whereas the expression of the presumably equally aboundant AtoC subunit was detected in the 2DE map of serotype-A CT by MALDI-TOF analysis.

CT08 (Hypothetical Protein)

CT082 (Hypothetical Protein) is part of an operon annotated as late transcription unit, and the expression of this ORF has been detected in the EB proteome. It is interesting that our data now indicate the likely exposure of the CT082 protein on the EB surface, supported by a relatively high K-S score (25.62) in the FACS assay. This localization together with its late expression in the replicative cycle suggests an important role of CT082 for some of the multiple EB functions. Surprisingly, we could not detect a sufficient infectivity neutralization mediated by our anti CT082 antiserum. However, as pointed out above, a negative results in a screening study is not to be taken as definitive because many factors (type of recombinant expression, quality of antibody response, the necessarily artificial conditions of the in vitro neutralization assay) may influence the outcome and affect the sensitivity of these assays.

CT398 (Hypothetical Protein)

The CT398 antiserum yielded the best neutralization titre in this study. The function of this hypothetical protein is unknown. However its presence in the EB proteome has been confirmed by mass spectrometry analysis. Our data now indicate its surface localization and neutralizing properties, and in silico analysis, although an N-terminal signal peptide is not detected by algorithms like PSORT, indicates the presence of a predicted coiled-coil structure between amino-acid residues 11 and 170 which is often present in bacterial surface proteins. Homology searches indicate some homology to a human muscle protein (MYST_HUMAN) and a structural similary hit with gi|230767|pdb|2TMA|A Chain A, Tropomyosin.

The negative results obtained in these studies are to be considered only negative in relation to the specific procedures and conditions adopted in the screening tests. That is, a negative result may simply be a function of the assay sensitivity. A typical example of such situation is represented by the recombinant porB protein (a conserved dicarboxylate-specific porin which may feed the *Chlamydial* TCA cycle) which in our hands proved to be surface exposed, in agreement with published data but unable to induce neutralizing antibodies. However, as shown by other workers in the field, porB is in fact also a neutralizing antigen. The discrepancy can be explained considering that the recombinant porB used in these studies. In order to display its neutralizing activity, the initially insoluble recombinant porB had to be refolded by extraction with 1% octlyglucoside and a dialysis step against PBS. Therefore, the neutralizing activity of porB clearly depends on its folding and in our screening work we may have obtained a recombinant porB with a folding which allowed the detection of surface exposure in the FACS assay but lost the neutralizing epitope(s). A similar situation could have been envisaged, from literature data, for the other known porin of *Chlamydia,* that is for the ompA gene product MOMP (CT681), the best studied vaccine candidate so far, which was also described as possessing folding dependent neutralization properties. Accordingly, one could have expected that in absence of specific refolding steps, our screening results could have failed to detect recombinant MOMP as

neutralizng. This however was not the case, and in fact the presence of MOMP within the short list of neutralizing antigens acquires in a way the value of an internal positive control.

The project described herein took advantage from previous work by selecting as a first option a number of *C.trachomatis* genes considered orthologous (up to 40% identity in the encoded polypeptide) to 'FACS-positive' genes of *C.pneumoniae*, i.e. to genes which when expressed as GST or (6)His fusion proteins elicited antibodies binding to purified *C.pneumoniae cells*. In Table 1(a) the names of CT proteins which had a corresponding positive screening results in *C.pneumoniae* are shaded, and it can be noted that 70% of the CT FACS-positive antigens we report have a Cpn ortholog previously described as FACS-positive. For general comments on the types of proteins so detected as potential constituents of the chamydial EB surface, and degree of expected agreement of these experimental finding with the current in silico annotations, we therefore refer the reader to the discussion of the previous results (Montigiani et al (2002) *ibid*). As far as the neutralization assay is concerned, the published Cpn work did not included this type of assay, however subsequent work from our laboratory identified in the FACS-positive set, at least 10 Cpn neutralizing antigens (Finco et al, submitted). It is noteworthy that the AtoS, ArtJ, Enolase and OmpH-like antigens (4 of the 9 neutralizing antigens identified in this study) when expressed as Cpn specific allelic variants have neutralizing properties for Cpn in vitro infectivity as well. In contrast with the precedent *C.pneumoniae* study, when the majority of the Cpn Pmp's yielded soluble and 'FACS-positive' fusion proteins, in the present study we obtained only 4 FACS-positive Pmp fusions proteins out of 9 Pmps identified in the CT genome.

Overall Summary

As *Chlamydia* infection is an intracellular infection, the currently accepted paradigm is that effective anti-*Chlamydia*l immunisation would require both an adequate T-cell response and high serum levels of neutralising antibodies and that "an ideal vaccine should induce long lasting (neutralising) antibodies and a cell mediated immunity that can quickly respond upon exposure to *Chlamydia*". Several sometimes contradictory studies have indicated that both CD4+ and CD 8 positive T cells have a role in *Chlamydia*l clearance (Loomis and Starnback (2002) Curr Opin Microbiol 5: 87-91). Indeed, there now appears to be a prevailing consensus that specific CD4+ T cells and B cells are critical to the complete clearance of intracellular *Chlamydia* and for mediating recall immunity to *Chlamydia* infection (see Igietseme, Black and Caldwell (2002) Biodrugs 16: 19-35 and Igietseme *et al* (1999) Immunology 98: 510-519).

It is well known that least two special types of T cells, CD4+ and CD8+ cells are required to initiate and/or to enhance CMI and and humoral responses. The antigenic receptors on a particular subset of T cells which express a CD4 co-receptor can be T helper (Th) cells or CD4 T cells (herein after called T helper cells) and they recognise antigenic peptides bound to MHC class II molecules. In contrast, the antigenic receptors on a particular subset of T cells which express a CD8 co-receptor are called Cytotoxic T lymphocytes (CTLs) or CD8+ T cells (hereinafter called CD8+ T cells) and they react with antigens displayed on MHC Class I molecules. Helper T cells or CD4+ cells can be further divided into two functionally distinct subsets: Th1 phenotype and Th2 phenotypes which differ in their cytokine and effector function.

Active Thl (IFN-gamma) cells enhance cellular immunity (including an increase in antigen-specific CTL production) and are therefore of particular value in responding to intracellular infections. Th2 cells enhance antibody production and are therefore of value in responding to extracellular infections (albeit at the risk of anaphylactic This enhancement of the Thl associated responses is of particular value in responding to intracellular infections because, as explained above, the CMI response is enhanced by activated Thl (such as, for example, IFN-gamma inducing) cells.

Such an enhanced immune response may be generally characterized by increased titers of interferon-producing $CD4^+$ and/or $CD8^+$ T lymphocytes, increased antigen-specific CD8+ T cell activity, and a T helper 1-like immune response (Th1) against the antigen of interest (characterized by increased antigen-specific antibody titers of the subclasses typically associated with cellular immunity (such as, for example IgG2a), usually with a concomitant reduction of antibody titers of the subclasses typically associated with humoral immunity (such as, for example IgG1)) instead of a T helper 2-like immune response (Th2).

The present invention demonstrates that combinations of CT antigens are proective against *Chlamydia* challenge. These CT antigenic combinations are capable of inducing both a antibody response (in terms of neutralising antibody) and a cellular mediated immune response (at least in terms of a Th1 cellular profiles) which can quickly respond upon exposure to *Chlamydia.*

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be covered by the present invention.

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Figure 1

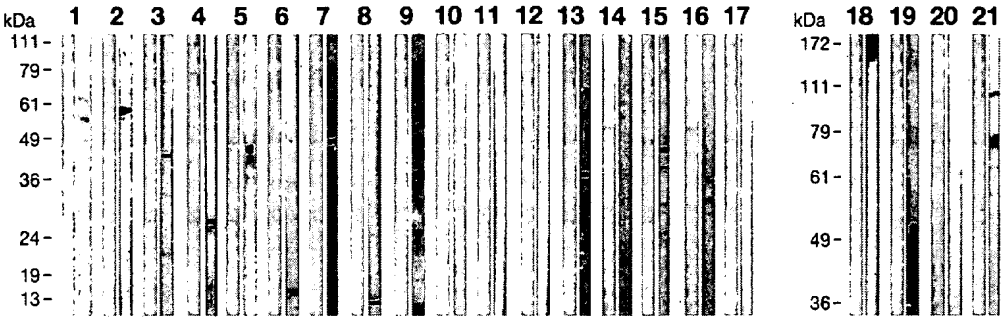

kDa
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
111-
79-
61-
49-
36-
24-
19-
13-
kDa
18 19 20 21
172-
111-
79-
61-
49-
36-

Figure 2

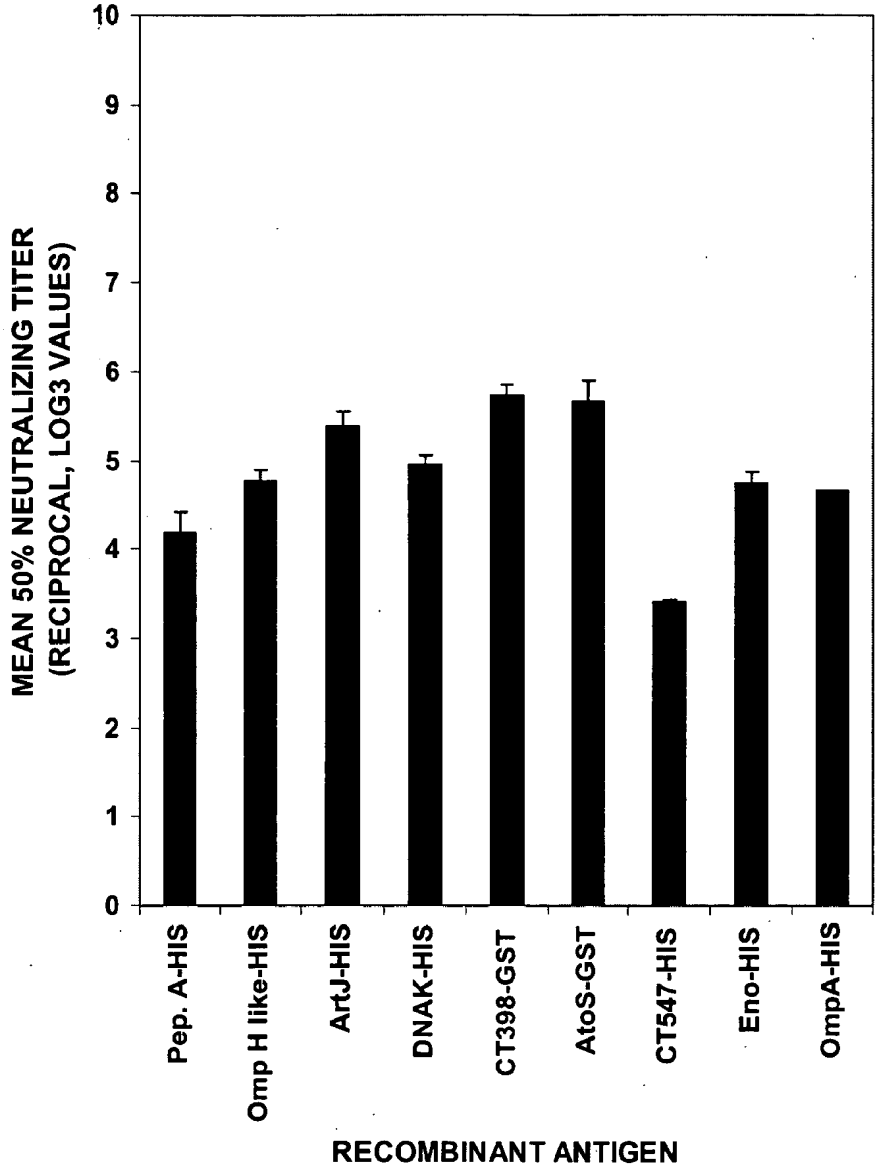

MEAN 50% NEUTRALIZING TITER
(RECIPROCAL, LOG3 VALUES)
10
9
8
7
6
5
4
3
2
1
0
Pep. A-HIS
Omp H like-HIS
ArtJ-HIS
DNAK-HIS
CT398-GST
AtoS-GST
CT547-HIS
Eno-HIS
OmpA-HIS
RECOMBINANT ANTIGEN

Figure 3

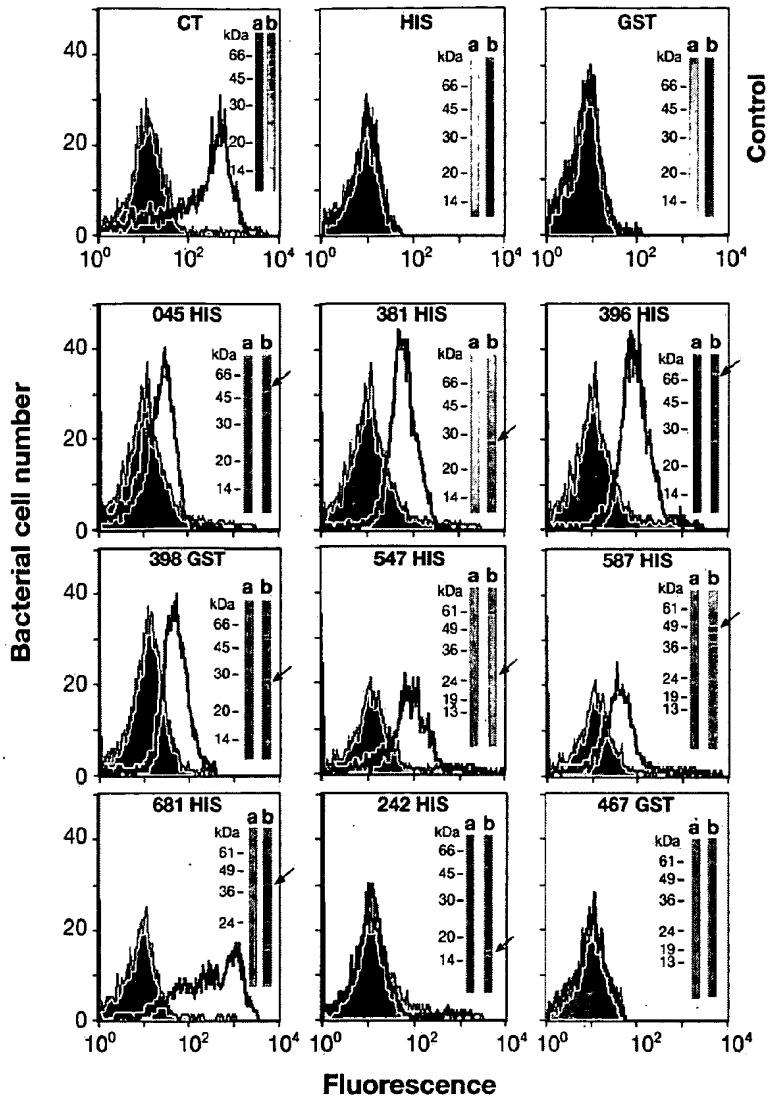

CT
HIS
GST
045 HIS
381 HIS
396 HIS
398 GST
547 HIS
587 HIS
681 HIS
242 HIS
467 GST
Control
Bacterial cell number
Fluorescence
kDa
a b

Figure 4

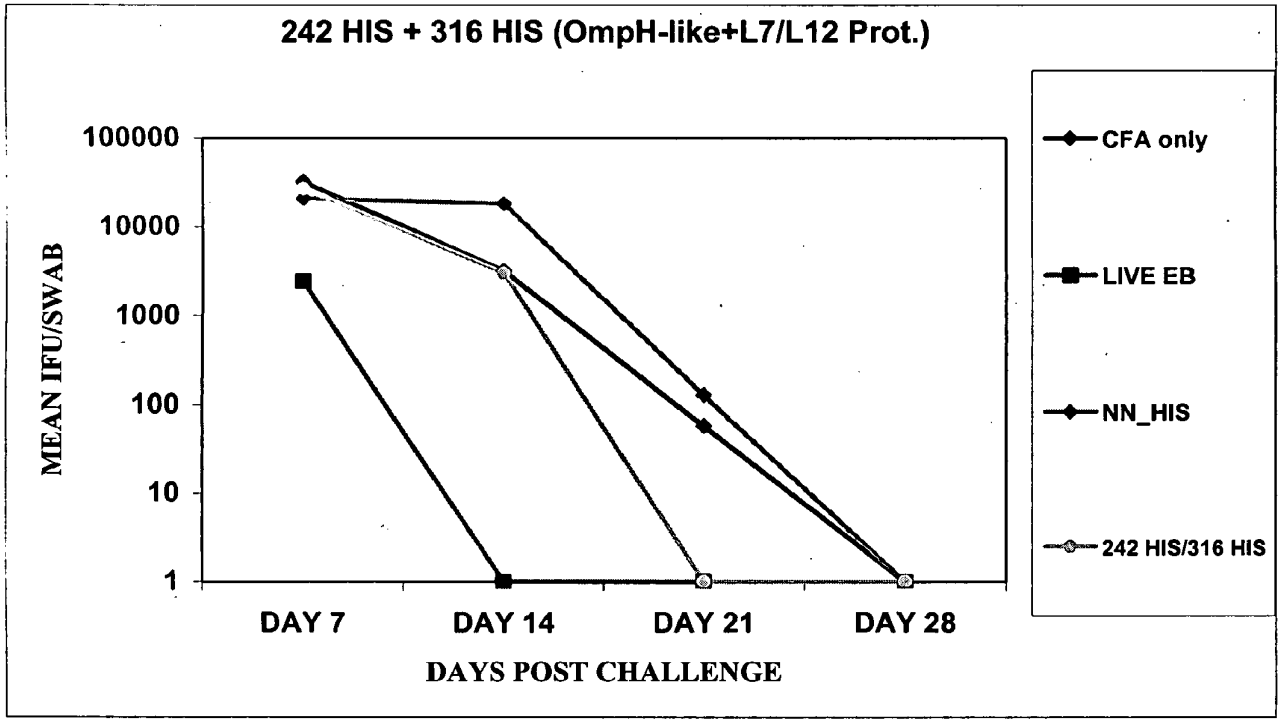
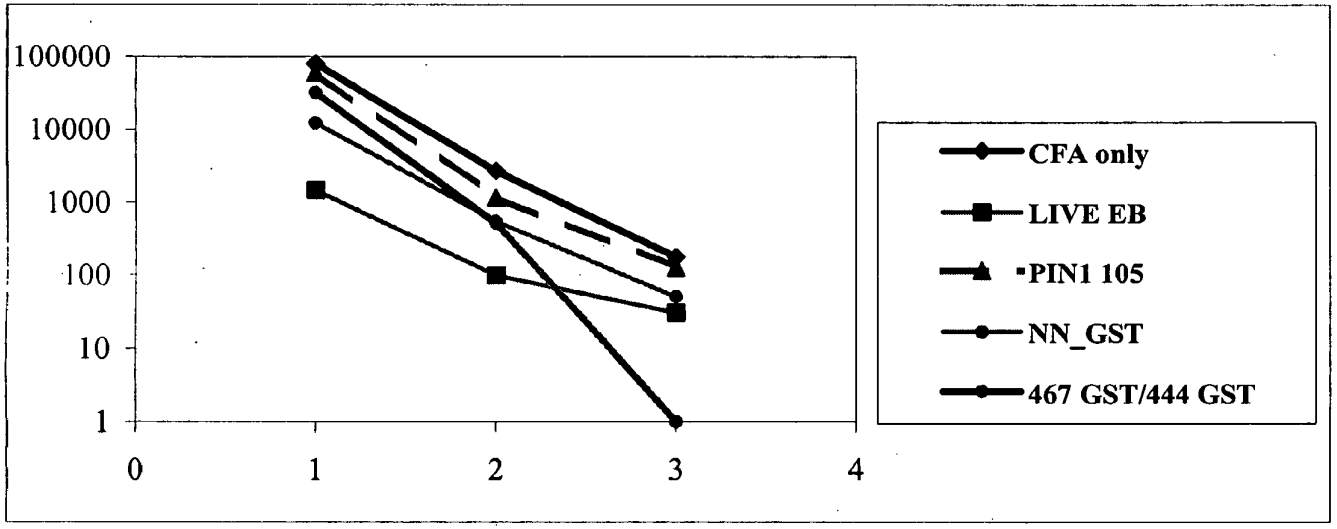
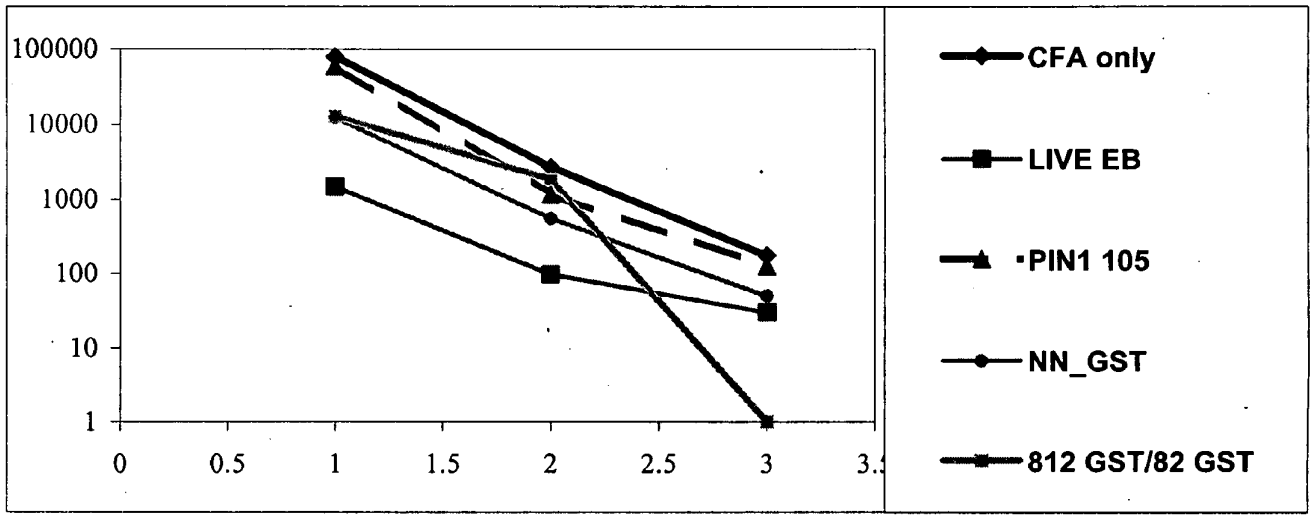

242 HIS + 316 HIS (OmpH-like+L7/L12 Prot.)
100000
10000
1000
100
10
1
MEAN IFU/SWAB
DAY 7
DAY 14
DAY 21
DAY 28
DAYS POST CHALLENGE
CFA only
LIVE EB
NN_HIS
242 HIS/316 HIS

Figure 5



*467-444
AtoS-9kDaCysRichLip

Figure 6



812-082
PmpD-Hyp.prot.

Figure 7(a)

Group	Day	Mean IFU/Swab	SD (Standard Deviation)	%IFU Reduction	P value
CFA only	7	4046	3200		
	14	1012	902		
	21	0	0		
live EB	7	50	73	99	0.07
	14	53	130	98	0.04
	21	0	0		
242+316 his	7	4740	3798	33	0.27
	14	127	178	87	0.02
	21	0	0		

Figure 7(b)

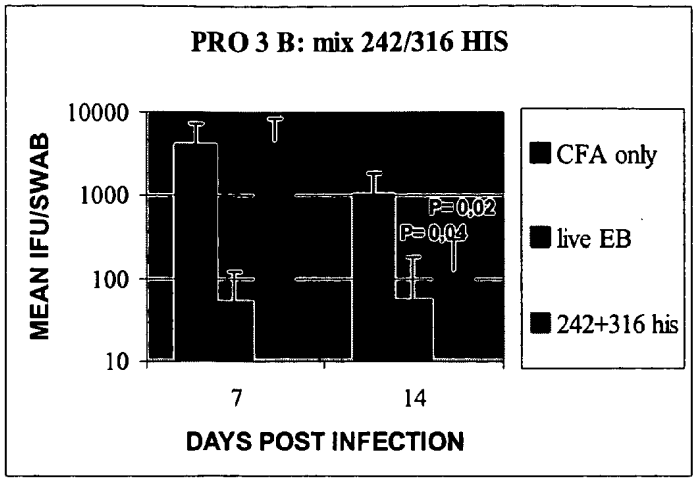

PRO 3 B: mix 242/316 HIS
MEAN IFU/SWAB
10000
1000
100
10
P=0,02
P=0,04
CFA only
live EB
242+316 his
7
14
DAYS POST INFECTION

Figure 7(c)

BALB/c MICE						
CT antigen	**ELISA TITER**			**W.B on EBs**	**FACS K-S**	**NEUT Titer**
	tot. IgG	**IgG1**	**IgG2a**			
242	24000	Not Determ	Not Determ	NC	15.5	01:50
316	10200	Not Determ	Not Determ	PC		

Figure 8(a)

GROUP	Mean IFU/swab	standard deviation	%Reduction
AlOH/CpG	2611	1631	
Live EBs	3	8	99
Mix5/AlOHCpG	93	140	96

Figure 8(b)

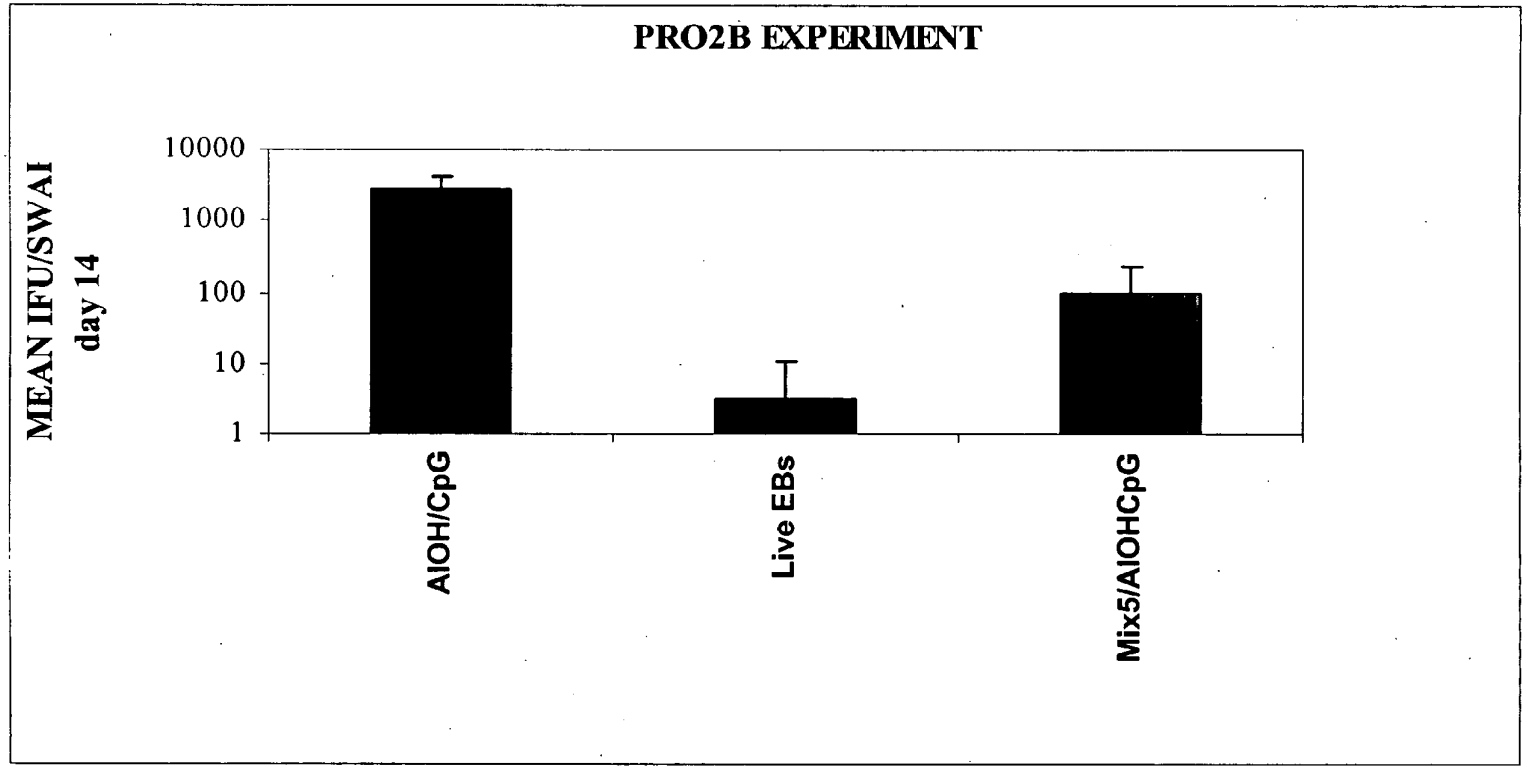

PRO2B EXPERIMENT
MEAN IFU/SWAI
day 14
10000
1000
100
10
1
AIOH/CpG
Live EBs
Mix5/AIOHCpG

Figure 8(c)

SEROLOGY DATA PRO 2B EXPERIMENT

BALB/c MICE										
CT antigen	ELISA TITER						W.B on EBs		FACS K-S	
	tot. IgG		IgG1		IgG2a					
	CFA	Al+CpG	CFA	Al+CpG	CFA	Al+CpG	CFA	Al+CpG	CFA	Al+CpG
45 HIS	62.0	123.000	273.000	384.000	12.000	73.000	P.C.	P.C.	32,34	33,55
89 HIS	37.000	360.000	360.000	400.000	38.000	66.000			"	"
396 HIS	84.000	129.000	203.000	380.000	53.000	144.000			"	"
398 HIS	61.000	113.000	318.000	552.000	34.000	117.000			"	"
381 HIS	134.000	16.000	129.000	17.000	42.000	8400			"	"

P.C.= **partially consistent** (a band of the expected M.W. is present together with additional bands of higher and/or lower M.W.)

Figure 9(a)

REDUCTION OF INFECTION DATA FOR THE 5 ANTIGEN MIX

PRO 4 EXPERIMENT

GROUP	DAYS POST INFECTION	Mean IFU/swab	SD	%Reduction of infection	P value
INFECTION	7	10112	5900		
	14	1048	1193		
	21	49	85		
AI/CpG	7	11810	10700		
	14	2460	2334		
	21	80	253		
CFA	7	6016	5475		
	14	920	2439		
	21	642	1912		
live EB	7	610	1764	95	0.002
	14	268	609	89	0.001
	21	0	0		
HIS +AI-CpG	7	12903	1067	NO RED.	
	14	4275	2677	NO RED.	
	21	807	1344		
45/89/396/398/381 HIS + AI-CpG	7	1668	1480	86	0.004
	14	555	909	77	0.02
	21	25	70	70	0.28

Figure 9(b)

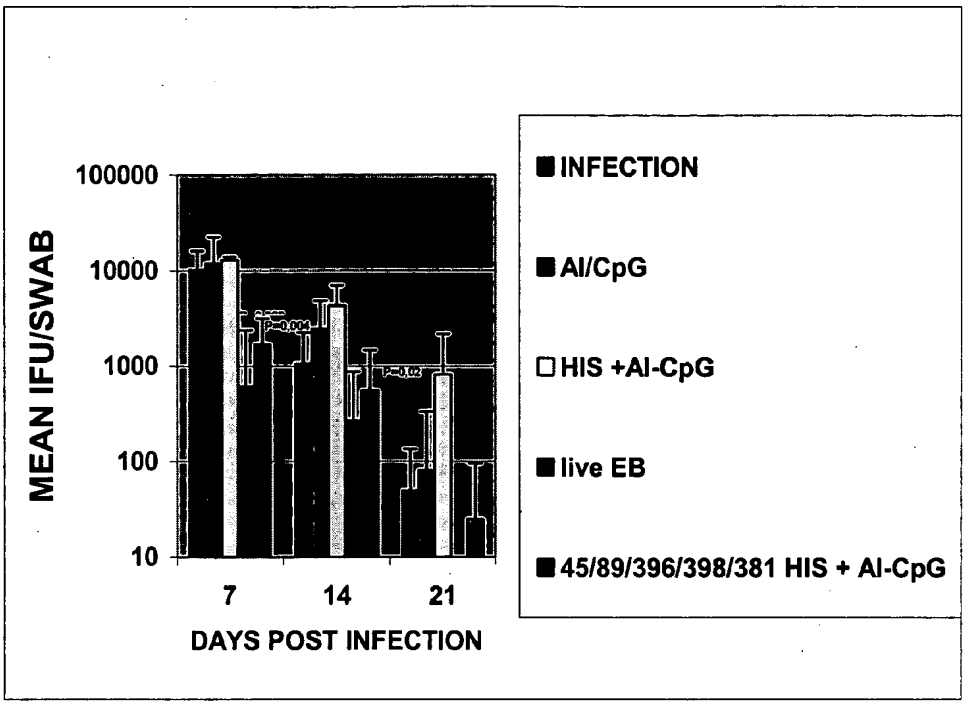

MEAN IFU/SWAB
100000
10000
1000
100
10
7
14
21
DAYS POST INFECTION
INFECTION
Al/CpG
HIS +Al-CpG
live EB
45/89/396/398/381 HIS + Al-CpG

Figure 9(c)

SEROLOGY DATA PRO 4 EXPERIMENT

BALB/c MICE						
CT antigen	**ELISA TITER**			**W.B on EBs**	**FACS K-S on Ebs**	**NEUT Titer**
	tot. IgG	**IgG1**	**IgG2a**			
45 HIS	121103	319524	60939	P.C.	27.10	1:120
89 HIS	75309	268048	58123	P.C.		
396 HIS	98131	291254	90791	P.C.		
398 HIS	49545	315410	64370	P.C.		
381 HIS	12034	23624	2910	P.C.		

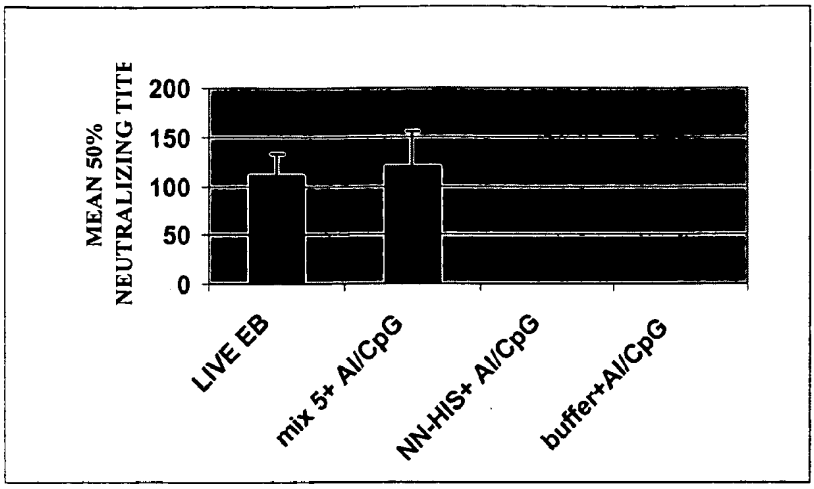
P.C.= **partially consistent** (a band of the expected M.W. is present together with additional bands of higher and/or lower M.W.)

Figure 10(a)

NEUTRALIZATION DATA FOR THE 5 ANTIGEN MIX

antigen	mean titer	SD
LIVE EB	110	22
mix 5+ Al/CpG	120	36
NN-HIS+ Al/CpG	0	0
buffer+Al/CpG	0	0

Figure 10(b)


MEAN 50% NEUTRALIZING TITE
200
150
100
50
0
LIVE EB
mix 5+ Al/CpG
NN-HIS+ Al/CpG
buffer+Al/CpG

APPLICATION DATA SHEET

Application Information

Application number:	To Be Assigned
Filing Date:	June 1, 2004
Application Type:	Provisional
Subject Matter:	Utility
Suggested classification:	To Be Assigned
Suggested Group Art Unit:	To Be Assigned
CD-ROM or CD-R?	None
Number of CD disks:	
Number of copies of CDs:	
Sequence submission?	
Computer Readable Form (CRF)?	
Number of copies of CRF:	
Title:	IMMUNOGENIC COMPOSITIONS FOR CHLAMYDIA TRACHOMATIS
Attorney Docket Number:	PP20662.004
Request for Early Publication?	No
Request for Non-Publication?	No
Suggested Drawing Figure:	
Total Drawing Sheets:	17
Small Entity?	No
Petition included?	No
Petition Type:	
Licensed U.S. Gov't Agency:	No
Contract or Grant No:	
Secrecy Order in Parent Appl.?	No

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State or Province of mailing address:	CA
Country of mailing address:	US
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Primary Citizenship Country:	Italy
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Middle Name:	
Family Name:	Grandi
Name Suffix:	
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State or Province of Residence:	
Country of Residence:	Italy
Street of mailing address:	c/o Chiron Corporation, P.O. Box 8097
City of mailing address:	Emeryville
State or Province of mailing address:	CA
Country of mailing address:	US
Postal or Zip Code of mailing address:	94662-8097

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Primary Citizenship Country:	Italy
Status:	Full Capacity
Given Name:	Giulio
Middle Name:	
Family Name:	Ratti
Name Suffix:	
City of Residence:	Siena
State or Province of Residence:	
Country of Residence:	Italy
Street of mailing address:	c/o Chiron Corporation, P.O. Box 8097
City of mailing address:	Emeryville
State or Province of mailing address:	CA
Country of mailing address:	US
Postal or Zip Code of mailing address:	94662-8097

Correspondence Information

Correspondence Customer Number:	27476
Name:	
Street of mailing address:	
City of mailing address:	
State or Province of mailing address:	
Country of mailing address:	
Postal or Zip Code of mailing address:	
Phone number:	

Fax Number:

E-Mail address:

Representative Information

Representative Customer Number:		

Domestic Priority Information

Application :	Continuity Type:	Parent Application:	Parent Filing Date:

Foreign Priority Information

Country:	Application number:	Filing Date:	Priority Claimed:

Assignee Information

Assignee name:	
Street of mailing address:	
City of mailing address:	
State or Province of mailing address:	
Country of mailing address:	
Postal or Zip Code of mailing address:	